

Vietnam—an inexpensive form of help

RECENTLY Dr Alastair Hay, a nutritional biochemist who has written frequently in our news pages over the past few years, went on a visit to Vietnam with financial support from *Nature*. The first of his three reports on postwar Vietnam appears in this issue. It comes at a time when Vietnam finds itself yet again involved in conflict, this time with its neighbour Kampuchea (Cambodia) over borders, although there is as yet no clear indication that the skirmishes will have a serious effect on redevelopment within the war-weary nation.

The scientific tradition has not been particularly strong in the past in this part of Asia; certainly not as strong, say, as in the Indian sub-continent or China. True, no country has ever had so much sophisticated technology dropped on it or so many large-scale and sinister 'experiments' performed in it, such as weather modification, the automated battleground, and defoliation. But all of this is of little value in postwar days and if Vietnam is to emerge as a healthy nation it will have to use its relatively few scientists intelligently and to give science a high priority in development plans. This it seems to be doing quite well.

But there are pitfalls along the way. The view has been expressed that since the army of a relatively small country was able to contain all the fury of the United States so might Vietnam's small band of scientists do remarkable things on their own. This is not, it should be said, a view expressed by the scientists themselves. But it has a certain superficial appeal and could do a lot to harm the development of good science within Vietnam. For there are already forces at work trying to make Vietnam stand on its own

feet economically—the USSR, the largest supplier of aid to Vietnam and also the country with the largest influence, is cutting back its aid in a drive to make the country more self-sufficient. If scientists, too, sense that they are being required to go it alone they could face grave difficulties.

For what Vietnamese scientists clearly need more than anything else—more even than gifts of expensive equipment, more than expressions of appreciation from politicians—is normal scientific relations with the rest of the world. This would mean a much greater flow of books and journals into the desperately under-equipped libraries and, even more, a flow of people into and out of Vietnam. And it is particularly important that Vietnamese scientists should not feel constrained to establish good relationships only with those in the West whose political position on the war was a matter of public knowledge. For sure, a small number of such scientists have done a lot of good for Vietnamese science, and have helped to raise Western awareness of the Vietnamese plight, but now the net has to be cast much broader to take in anyone who might make a significant contribution, whether or not they are politically committed.

The largest obstacle to the development of science in Vietnam is an information barrier—not deliberately erected but just a natural consequence of war and isolation. It needs a concerted effort by all scientists of good-will to dismantle it. In Britain the Ministry of Overseas Development, the British Council and the Royal Society are all in a position to make a contribution to this relatively low-cost way of stimulating science. □

Holiday diary

January 7. To the misnamed Science Museum at South Kensington with the children. After the tide of irrationality and anti-science of the past decade, good to see the place filled with youngsters still interested in logical ways of thinking. Sure, most of them will remember the place mainly for the knobs to press and the handles to turn, but even so something probably rubs off on them of fascination for machinery and orderliness. 'Please keep off the moon', says the lunar exhibit. Lasers all the rage these days; one on the roof of the Royal Academy, of all places, to advertise a show there; one used at Covent Garden in Tippet's new opera; so off to top floor to see Science Museum's own Laser exhibition, even though pricey to go in (75p). Quaint

and dubious distinction between 'laser light' and 'ordinary light' on a display. Lots of good honest mundane uses of lasers—communication, pipe laying, surveying, cutting cloth and so on. Striking Russian holograms of museum-pieces. Children a bit bored because not many buttons to press. Quite a surprise to get to the end of the show so quickly, though children clearly mildly relieved as they can get back to the working models. Holograms on sale at the exit, for £12.50 each. But what do they portray? 'That's Paul Revere', says man at desk. 'Who?', says my neighbour. 'Haven't you anything else?', he asks, 'Yes—we have all the astrological signs too'. Rapid exit to the irrational world. □



Disaster relief needs more research

John Rivers, of the London Technical Group, argues that more research on disaster relief is needed if funds are to be used effectively*

THE CYCLONE and tidal wave that struck Andhra Pradesh in November last year killed about 30,000 people. But it devastated half a million, and made 2,000,000 more homeless. In the previous two years cyclones had also struck the state, ruining the harvest and leaving farmers deeply in debt. So, at the start of 1978, Andhra Pradesh is poised between recovery and a collapse that would make it rank alongside Sahel and Ethiopia in the vocabulary of suffering.

The immensity of this disaster has not, however, been reflected in the interest shown in it by the rest of the world. It has been a minor story, a few column inches on an inside page, while UK headlines have concentrated on the firemen's strike and the value of the dollar. Few seem to care about Andhra Pradesh. USAID figures, issued one week after the cyclone, estimated that aid from the international community was less than 1% of the \$675,000,000 of damage done. In Britain, the Disaster Emergency Committee's Appeal was a flop, raising only a quarter of the desired million pounds.

The public's lack of interest may not be surprising. The media have produced an unending diet of catastrophe. And the public may be becoming cynical about the usefulness of aid. This would be understandable, given such misdirected aid as the sending of skis to flood victims or brassieres to the starving, which rightly get pilloried in the press. But though newsworthy such events are of minor importance. The major waste in relief funds is more subtle.

In the rare cases where disaster research has been undertaken, the research has exposed the mythological basis of traditional relief. Inoculation campaigns, for example, have been an expensive component in all flood relief programmes since the war, as they were in Andhra Pradesh. Yet epidemiological studies by the Centre for Disease Control, Atlanta, Georgia, have revealed them to be unnecessary and ineffective. The vaccines are not as efficient as is often supposed, and they take about 10 days and two or more inoculations to give a reasonable level of immunity. Moreover the dilution of sources of contamination is much greater, and disruption of water supplies much less in disasters than has hitherto been supposed. So the alternative of simple symptom-reporting systems and intensive investigation of supposed outbreaks, is, as the Guatemalan experience has shown (Romero, A. B., Cobar, R., Western, K. A. & López, S. M., *Disasters* 2, 19-26; 1978) cheaper and more efficient than the fabulously expensive vaccination campaigns that hypnotise relief agencies. The money wasted on such unnecessary flummery is unlikely to have saved lives in Andhra Pradesh; almost certainly it cost lives by diverting scarce resources from more useful action. Other examples of such re-evaluation could be cited, even more await study. As long as disaster aid is less than adequate there will be a need for a science of relief, for a disaster technology.

In the days following the Bengal cyclone in 1971, the London Technical Group (LTG) was started by a group of postgraduate students anxious to encourage the growth of this field. Our first inclination was to initiate a dialogue between aid administrators and academic scientists. It was a twofold failure. First, most academic scientists had a crucial problem in their lack of practical experience of disaster situations. Scientific expertise, no matter how exalted, could be proved inappropriate by peculiarities, often

social, of the population affected by the disaster. Food aid, for example, is a fairly unchallenged item of disaster relief, being sent on the premise that if infantile malnutrition is endemic in good times, it must reach horrific proportions following a disaster. In Andhra Pradesh, as in the Bengal Cyclone, this is unlikely: the sea surge itself killed the very young and the very old, so that the malnourished and vulnerable died, and only the fittest survived. The aid sent presupposed high levels of infantile malnutrition; they are probably lower than ever before.

In housing too the social factor can negate apparently good logic, and the whole field of disaster shelter is littered with clever designs that nobody wants as homes. There is a considerable body of evidence that the best choice for disaster shelter is to use local materials and local labour to rebuild. The result is acceptable, familiar, and morale boosting (Davies, I, *Disasters* 1, 82-90; 1977). Most architects and designers, however, seem to be unaware of these constraints, and find ready sponsors for their designs in government and charitable disaster relief agencies.

This pattern is repeating itself in Andhra Pradesh where the state government and some voluntary agencies are erecting pre-stressed concrete buildings ill-suited to the climate but enough of a status symbol to lead to consumer resistance against the modifications of traditional buildings that are being offered by other agencies. This indeed is typical of the second reason for LTG's failure:—the unwillingness of voluntary agencies and governments to accept scientific evidence, where this points to the need for a change in accepted practice.

In view of this failure it became apparent that if scientists are to contribute to disaster research, there must be a re-assessment of the roles they play.

●First, acknowledging the limitations of the individual expert advisor, we should set out to develop multidisciplinary groups who can meld their individual talents into a new discipline of disaster research. UNESCO already has its earthquake team which visits the site of earthquakes for seismic research. There is no reason why analogous teams, independent of aid donors, should not be formed to investigate the social and medical impact of natural disasters. Research into the effectiveness of disaster aid could be immediately undertaken if only the aid agencies would agree to fund it. It would provide valuable evidence about any need for change in aid policies. In time, if such research became routine, results would be available to relief agencies to direct aid in any current disaster, rather than on a retrospective basis.

●Second, there is a need for longer term investment in research into the nature of disasters themselves. There is much we need to know about disasters, and the social response to them. Scientists as yet may have only an impersonal understanding of disasters, but at least they do know how to ask questions.

As always with research the problem is one of money, and investment in a programme of disaster research requires an act of faith by some funding agency. Justification for such faith may be found in the surprising yield of results from the fragmentary funding the subject has received in the UK and USA over the last 10 years—sufficient indeed to support two journals specialising in the field.

What can science achieve? Eloquent testimony exists in 'Maladie de Famine', a little known book of researches into the pathophysiology of famine which, although over 30 years old, is still the definitive work on the topic. It was a study conducted in the Warsaw Ghetto, by scientists, themselves victims of starvation and under sentence of death. No better memorial could be found to these men than to continue their work. □

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Science helps to rebuild Vietnam

Vietnam believes science to be the key to economic recovery. She hopes to have a "modern scientific capability" within 15 years. Alastair Hay reports

ONE of the longest wars in this century, the war in Vietnam, finally came to an end on 30 April 1975. It had claimed over a million lives and was fought for the best part of thirty years.

Few countries can have featured in as many television documentaries as Vietnam. For many of those watching, the image of Vietnam was fashioned by the television screen; it left a memory of a battered people and scarred countryside.

This is the legacy of war. Vietnam faces innumerable problems as she begins her programme of reconstruction. The problems in the health field illustrate the extent of the damage. The World Health Organisation cites figures from Vietnam of one million people infected by venereal disease, about 300,000 prostitutes, and half a million drug addicts, in a population of some 50 million. Endemic malaria affects some 18 million people in Vietnam. And the annual tuberculosis mortality rate is 30 per hundred thousand—the highest in the western Pacific.

Agricultural problems are just as daunting. Vietnam feeds 50 million people, but only farms 5 million hectares. Comparable figures for Eastern Europe cited by Vietnamese agriculturalists are estimated at a ratio of one person per hectare. If Vietnam is to become self-sufficient in food production—which is her intention—her tillage has to increase, and the efficiency of crop cultivation must improve.

But two obstacles impede her progress; unexploded weapons and bad weather. Literally hundreds of square miles of agricultural land have to lie fallow because of the presence of unexploded bombs and mines; mine-sweeping—currently being undertaken by the army—is required before the land can be pronounced safe.

The weather, too, has had disastrous consequences for the last two rice harvests. First it was too dry and then too cold. Yields have been too low to meet home demand and monthly rice rations have had to be reduced from 18 to 14 kilograms per person per month. To make up the remainder of the shortfall, scarce foreign currency had to be used to purchase rice on the international market. That currency was to have bought 3,000 tractors to aid the mechanisation of agriculture. In the event only 500 could be



Suffering from the ravages of war

acquired, all of which are in use in the Mekong Delta—the country's most fertile region. 500 tractors for a population of 50 million people can hardly be expected to revolutionise Vietnamese agriculture.

It is against this background that the scientists in Vietnam are having to orientate their work. They may lack the necessary material resources to tackle many of the problems confronting the country, but they have no lack of prestige. Science is seen by the Government as the key to Vietnam's economic recovery. Science and technology are important enough to warrant a spokesman in the Politburo—the highest policy making body. The minister with this responsibility is General Vo Nguyen Giap.

Giap is not well known in the scientific world. As his title might suggest, his has been a military career. Giap's claim to fame is as victor of Dien Bien Phu, the battle which in 1954 signalled an end to French involvement in Indochina. He has had a number of other responsibilities before assuming the portfolio of Minister of Defence, a post he still retains.

Vietnam's scientists were not displeased when Giap was nominated to deal with their problems. They find him an able minister and effective spokesman. To have one of the seven members of the Politburo—and a possible future prime minister—as their

representative is a situation any lobby would welcome.

Much of Vietnam's basic research is done in the Vietnamese Scientific Research Centre (VSRC) in Hanoi. The centre, now nearing completion, is part of the Soviet Union's aid programme to Vietnam. It will eventually house over 1,000 scientists, technologists and technicians in its four component institutes: those of biology, chemistry, physics and earth sciences. Two additional institutes (of oceanography and experimental biology) are located in the south of the country in Ho Chi Minh City (formerly Saigon).

Dr Nguyen Van Dao is the most senior of the seven vice-chairman of the VSRC; in effect he is its director. In an interview made easier with numerous cups of tea, many bananas and sweets he outlined his centre's research programme. The centre is, he says, "the main research institution in Vietnam". It also controls some Government programmes. One of the most important, says Dao, "is an investigation of our natural resources". Dao considers this knowledge to be vitally important for a developing country. The scientists in the centre will be surveying the coastal seabed as well as mapping land resources. Dao adds that many Vietnamese scientists

Alastair Hay was recently in Vietnam as a Nature travelling fellow. He was supported in part by the London Technical Group.

suspect that the United States could have valuable satellite pictures which would aid the surveys they propose to make. He acknowledges, however, that this information will probably be classified, but maintains that the Vietnamese would make better use of it than the Americans.

The VSRC, says Dao, is really only starting its research programme; its responsibility will increase in the future. Emphasis is currently placed on the problems of agriculture and medicine; on developing the country's electronics industry; and on establishing geophysics and hydrodynamics on a sound basis. "Our aim" says Dao "is to have a modern scientific capability in 15-20 years. This is a great responsibility. We are virtually starting from scratch after 30 years of war and at a time when the country is so short of resources". Dao would welcome extensive collaboration with Western scientists and scientific institutions. This, together with donations of scientific literature and equipment, could help Vietnam meet her twenty year programme. Dao expressed his appreciation for the aid the centre has received from other socialist countries, but adds that there is a desperate need for much more help.

On a tour of the centre it is apparent that some subjects are better provided for than others. Physics is generally acknowledged to be an expensive science, chemistry less so, but costly nevertheless. Historically this is how resources have been apportioned in Vietnam for both physicists and chemists were needed in the war effort. Biology and the other sciences all trailed behind.

The Head of the Institute of Biology, Dr Tuoc, has hopes that this situation is about to change in view of the Government's stated view that agriculture must be accorded more attention. Tuoc emphasises that the VSRC is concerned with basic science, but that this will have a practical application in the future. Some biologists in his Institute are engaged on plant breeding programmes with rice seeds obtained from the International Rice Research Institute in the Philippines; their aim is to produce a crop with a higher nitrogen content and to find a rice variety that will withstand a colder environment. Others are studying diseases of rice and tobacco—two of Vietnam's most important crops. Some of the research in vitamins in progress will include a compilation of these nutrients in the country's foodstuffs. Zoologists are similarly engaged on a programme of cataloguing Vietnam's fauna. The two rooms allocated to them in the Institute of Biology are filled with animal specimens destined eventually



Alastair Hay

Zoological specimens await attention

for a museum of natural history.

Of the four institutes in the VSRC, physics is by far the best equipped. The physicists have some real successes to their credit, and are perhaps the most confident of Vietnam's scientists. Professor Nguyen Van Hieu is a vice-chairman and in charge of the Institute of Physics. Open, warm and very forthcoming, Hieu is a perfect ambassador for Vietnamese science. Hieu is a high energy physicist and a professor at the Dubna high energy physics institute near Moscow. His institute has strong ties with Dubna and some Vietnamese physicists are currently engaged there on 1 to 3 year contracts. Many of the physicists—like most of the other scientists in the centre—are either trained in the Soviet Union or Eastern Europe; some studied in France and Germany.

The real achievement of Hieu's institute is the completion of a five-year programme to establish Vietnam's electronics industry. With many patents and raw materials bought from the West the physicists now produce their own transistors. It has proved to be an expensive undertaking, but well worth the financial support provided by the grants from industry. Vietnam's technologists have also worked on the electronics programme for its duration and in preparation for the transition of the work from pilot stage to full scale production. 1978 is destined to be the year of the first Vietnamese radio components.

To offset the cost of the transistors the physicists—in co-operation with the earth scientists—are actively studying the properties of Vietnam's natural resources. They hope to find the necessary raw materials to assist with

manufacture. In the past most of the research of the institute was of an applied nature; only recently has it been possible to do basic research. The six departments in the physics section cover a variety of topics. In theoretical physics, there are quantum field theorists, and solid-state theorists. In other departments some study semiconductor physics; some optics—for prism and lens manufacture to study the optical properties of semi-conductors and solid state materials—and quantum electronics; some work on technical physics for physical measurement; some on the physics of radiation; and some on crystallography.

The chemistry institute is headed by Dr Khoi Nguyen Huu. Vietnam's chemists are also engaged on a study of the country's natural resources. Their interest is centred on plant terpenes, steroids and alkaloids. In co-operation with botanists, plants used in traditional medicine are being grouped according to their chemical taxonomy. Eventually the active ingredients in the medicaments will be identified and synthesised. Certain stages of the process are feasible at the moment, but for positive fingerprinting all chemicals still have to be sent to foreign laboratories, a process the chemists find very time consuming.

The chemists are also studying the oil resources in Vietnam. The final aim of the programme is to assist industry to extract commercially important oils from anise, citronelle, cinnamon, basilicum, dugenol and mint. With further departments for inorganic analysis in Hanoi, and others for oil chemistry and organic synthesis—mainly polymer chemistry—in Ho Chi Minh City the chemists have the necessary manpower for their work. Again the real problem is a chronic shortage of equipment and of available chemicals and solvents.

The VSRC is the best equipped of Vietnam's research institutions. Its programme is closely allied to the country's needs. But like all of Vietnam's research institutions it lacks many resources. Scientists, scientific institutions and governments in the West could do a great deal to assist the development of science in Vietnam. The aid which is currently given to Vietnamese scientists by Western donors is still far from adequate. Far more in the form of literature, equipment and exchange facilities for scientific co-operation is required.

When Vietnamese scientists are so obviously keen to make contact with their Western counterparts it surely is an opportunity to be grasped. A generous response from the West would be in order, for Vietnam's burden is one few would wish to carry. □

US establishes new directorate for applied research

IN a renewed attempt to develop an effective mechanism linking scientific research to national priorities, the National Science Foundation (NSF) announced last week that it is to set up a directorate for applied science and research applications (ASRA).

The new directorate, with an initial annual budget of about \$60 million will replace the Research Applications Directorate, which has been responsible for the increasingly-criticised programme of Research Applied to National Needs (RANN) set up in 1971 in an attempt to bridge the gap between basic research and its applications.

The main difference between RANN and the new directorate is that whereas the former was primarily organised around individual problems, ASRA has been organised on more general principles.

Thus the RANN programme has at present five separate foci, the three most important being resources, environment, and the loosely-defined field of 'advanced productivity research and applications'. In contrast, ASRA will be divided into six functionally-defined units, including an office of problem analysis, a division of integrated basic research, a division of applied research, and a division of problem-focused research applications.

In addition, whereas RANN operated relatively independently of other research programmes financed by the NSF, ASRA will work closely with the foundation's three directorates for basic science: for example it will help to identify and subsequently stimulate basic research relevant to agreed goals.

In the US, the NSF was placed on this path by a 1969 amendment to its original act under which the foundation was authorised to "support, through other appropriate organisations, applied scientific research talent relevant to national problems involving the national interest".

In line with this amendment, and with President Richard Nixon's emphasis in a State of the Union message on harnessing the "discoveries of science in the service of man", the RANN programme was set up with responsibilities that included the identification of unrecognised research needs, and increasing the effective use of science and technology in dealing with national problems.

Despite stimulating a number of important research developments—for example into the use of geothermal and solar energy—the organisational basis

of RANN has in recent years encountered growing criticism, reflected in a gradual fall-off in its budget appropriation.

RANN faced the dilemma that if it defined research areas too narrowly, then many research proposals would not fit; but if the definitions were too broad, it was accused of encouraging a 'shotgun' approach to problem-solving without any clear description of research objectives.

Further criticism came from the scientific community, who objected to the apparent attempt to impose unreasonable goals on research efforts. In a highly critical report on RANN's research efforts in the applied social sciences, for example, the National Academy of Sciences claimed that these projects were "of highly variable quality and, in general, not impressive".

Although the Research Applications Directorate introduced a new structure for the RANN programme in 1976, unease remained, and in December that year a task force was set up under the chairmanship of Dr John Whinnery of the University of California, Berkeley, to 'review and advise the director on science applications across the Foundation'.

Dr Whinnery presented his report last summer, suggesting a number of alternative structures, from which the new directorate for applied science and research applications was selected by the NSF. The directorate, which comes into effect next month, is divided into six units as follows:

- The office of assistant director, which will carry out the policy-making, management, review and co-ordination functions of the directorate;
- The office of problem analysis, which will work with internal NSF and external groups and organisations in assessing problems for strategic and programmatic planning;
- The division of integrated basic research, a new section which will provide a direct link between ASRA and the basic research directorate of NSF by jointly identifying basic research related to significant national problems;
- The division of applied research, whose two sections—one dealing with applied social and behavioral sciences and the other with applied physical, mathematical and biological sciences—will support research proposals benefiting social, economic and technical problems and policy issues, as well as identifying and stimulating the growth of new technologies and processes

based on discoveries in various fields of science;

- The division of problem-focused research applications, which will support the application of scientific and technological capabilities to selected problems of society which are of critical national importance. (This division will initially contain four research programmes: earthquake hazard mitigation, chemical threats to man and the environment, biological alternatives for industrial feedstocks, and community water management); and

- The division of intergovernmental science and public technology, responsible for integrating science and technology into federal and state planning.

The head of the new directorate will be Dr Jack T. Sanderson, who took over the Research Applications Directorate last summer on the resignation of Dr Alfred J. Eggers, RANN's first director and now director of Lockheed's Palo Alto research laboratory.

Dr Sanderson claims that part of the new directorate has responsibility for "the most complicated part of the research spectrum" in contrast to the organisation of basic research, with its peer group system and its established paradigms. Linking research to national priorities is, he admits, an area in which the necessary mechanisms are "least well understood".

The stakes are high; no one doubts that there is a close relationship between basic research and technical development—but no one has yet come up with a totally convincing (and effective) way of pinpointing precisely how support for the former can stimulate the latter.

Will ASRA succeed where RANN seems to have failed? According to Dr Sanderson, the organisation of the new directorate is based on various models, each of which has already demonstrated a certain success, thus the integrated basic research division is modelled closely on the NSF's energy-related programme, instituted in response to the energy crisis of 1973. Under this programme NSF officials and research scientists together identified 40 priority research topics for which extra funding was subsequently made available. "Similarly the model for the problem-focused research applications division was developed after looking at the way applied research activities are carried out in government and private industry" Dr Sanderson says.

So far, the reaction to the new directorate from Capitol Hill, from which some of the most vocal criticisms of

RANN were heard, has been a cautious welcome. But President Carter is known to be carrying out assessment of the effectiveness of the current organisation of research funding. A \$4 million programme providing basic support

for university research, known as "basic research stability grants", has already been "impounded" by the President from the NSF's 1978 budget prior to the outcome of this assessment. ASRA will therefore no doubt

receive close attention from both the House and the Senate during the hearings on the President's proposals for the science budget, due to take place in March.

David Dickson

Shcharanskii may soon be brought to trial —without a lawyer

THE US National Academy of Sciences has made an unprecedented demand for permission to send a legal observer to the forthcoming trial of Anatolii Shcharanskii, the Soviet cybernetician, who, until his arrest last March acted as spokesman for the unofficial 'Sunday Seminars' and was an active member of the Moscow Helsinki Monitoring Group.

Since his arrest, Shcharanskii has been held incommunicado, pending investigation on charges amounting to treason. The official 9-months investigation period expired in December, whereupon the investigating officers requested and were granted an extension of six months. Nevertheless, Shcharanskii's mother has now been told that she should find a lawyer for her son, not later than Friday, January 13, 1978—a somewhat ironic instruction since already at least 30 Moscow lawyers have refused to act in his defence.

According to a TASS statement of 22 December, the charge against Shcharanskii is that of giving assistance to a foreign State by systematically supplying his 'masters' with slanderous information about the Soviet Union, which was then actively used for ideological diversion against the Soviet Union, and supplying to the West information about Soviet enterprises and institutions, data which constituted official secrets. Already quasijudicial 'hearings' in Stockholm, New York, Paris and London, have presented a considerable bulk of evidence in rebuttal of these charges. While the publicity value of such moves may be considerable, the comment of *Literaturnaya Gazeta* that it is only a court which can determine Shcharanskii's innocence or guilt and that it is not for US lawyers to maintain law and order in the USSR does have a certain justification. The move of the NAS, in requesting the presence of an observer at the trial, is possibly proving more difficult to answer, since, although the hand-delivered letter was accepted by the Soviet Embassy in Washington, to date no answer has been received—a standard Soviet practice in dealing with embarrassing requests from abroad.

The NAS appeal was made by its



Anatolii Shcharanskii: held incommunicado

Committee on Human Rights, which was founded in 1976 at the requests of the grass-roots membership of the Academy. The Committee includes some 50 members of the Academy and in all, out of a membership of 1,200, over 350 members have done active work on behalf of dissident or imprisoned scientists. To date, their most notable success has been the release of the Argentinian Juan Carlos Gallardo, although they freely admit that the credit for this must go not to the NAS alone but to the whole human rights movement.

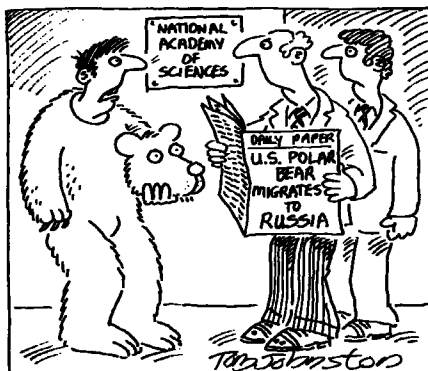
The Shcharanskii appeal noted the "widespread reaction . . . and grave concern" among Shcharanskii's colleagues in the USA and elsewhere, and suggested that further action against him would "severely damage scientific interactions with the USSR". This is not an idle threat—already the American Association of Computing Machinery has threatened to break off exchange relations with the Soviet Union over the Shcharanskii case. The Human Rights Committee of the NAS acts essentially as a mouthpiece of membership pressure. If there should be a widespread demand for a severance or suspension of such agreements

(and with more than a quarter of the membership actively involved in human rights activities, this is by no means impossible), the implications would be considerable. Although there exists a direct intergovernmental scientific exchange programme (established by the Nixon-Brezhnev talks of 1972), the exchange agreement between the Soviet and US Academies is a much older one (dating from 1959) and is both extensive and prestigious.

The NAS appeal is only one expression of the unprecedented concern which the Shcharanskii case has evoked. A number of factors are involved. One is, of course, the gravity of the charges. The choice of these charges, and suggestion of CIA involvement may in itself be a result of human rights action in the West. A few years ago, when intervention by such bodies as Amnesty International on behalf of dissidents became embarrassing, certain attempts were made to produce criminal charges so as to put the case outside Amnesty competence. Thus the refusenik physicist Viktor Pol'skii was accused of dangerous driving and manslaughter. The human rights activists promptly reformulated their terms of reference to include such cases, so that

from this point of view, the Shcharanskii case may be envisaged as the next move in a grim kind of human chess. Incidentally, the charge of disclosing state secrets is particularly ironic, since on graduation Shcharanskii carefully avoided taking a post where he might be exposed to classified information, which could hinder his chances of emigrating to Israel. Similarly, the Kiev physicist Vladimir Kislik, against whom, it is feared, similar charges may be in preparation is specifically accused of illegally sending abroad a scientific paper for publication. Not only is the information contained in it not secret; according to a western referee, the subject matter is so well known that he would not himself recommend publication.

Shcharanskii is somewhat of an exception among the refusniks, who, while awaiting their visas for Israel generally try to avoid dissident politics. The usual refusnik policy, as explained to *Nature* by Mark Azbel, former leader of the seminar is to avoid any confusion of the two issues, lest the



"Gee prof, there must be easier ways of getting in to observe the Shcharanskii trial"

would-be emigres be accused of subversion and the dissidents of Zionism. Whether such a distinction is possible is a moot point, since the Soviet authorities themselves seem bent on confusing it, by claiming, for example, that Sakharov is "really a Jew called Zuckermann". Shcharanskii, however, during his time in 'limbo' took an active interest in the Helsinki monitor-

ing group. Thus his arrest can be seen either as part of the renewed pressure on the Seminar, the 70 hard-core members of which are now being subjected to increasing surveillance including the use of cars with special listening devices, or as part of the campaign against the Helsinki monitoring groups two of which (in Ukraine and Armenia) had several members arrested over the (Western) Christmas holidays, when, it was presumably hoped, the news might pass unnoticed abroad.

From its very beginning, the Soviet human rights movement has been predominantly a movement of scientists, and this fact has undoubtedly contributed to an increasing concern about Western scientists with such problems. To date, except in the special case of psychiatry, where misuse of professional knowledge for political ends was involved, there has so far been no threat that protest abroad would lead to the severing of scientific relations. How far such sanctions will go in defence of Shcharanskii remains to be seen.

Vera Rich

Tory attacks energy gap forecasting—and the fast breeder

A BRITISH Conservative MP has attacked the hypothesis that there will be an 'energy gap' in the UK towards the end of this century, and questioned the economic sense of a commitment to a large programme of fast breeder reactors. The MP, Mr Nigel Forman, is the author of a Conservative Political Centre (CPC) pamphlet published this week titled *Towards a more conservative energy policy*. The document, while not a formal statement of Conservative Party policy, is according to a CPC spokesman "fairly close to the mainstream of Conservative thinking".

Mr Forman calls for emphasis on improving the efficiency of the production and distribution of energy, where 30% of primary energy is wasted, and proposes a flexible energy policy which operates on the demand as well as the supply of energy.

In a refreshing document which seems to verge on the radical rather than the conservative, the MP calls for more research on renewable resources, and for an approach to energy policy which will "liberate it from the requirements of the major vested interests". The conventional wisdom on renewable energy sources "seems to assume that they will make only a limited contribution to the energy supplies of the UK by the year 2000". However the main reason that the predictions are modest "is that until very recently

the R&D effort on nuclear energy, for example, exceeded that on all the renewable resources together by a factor of about 100".

Mr Forman brings his strongest criticism to bear on conventional energy forecasting and on the economics of the fast breeder programme. "It is when the experts begin to foretell the future that the trouble really begins" writes Forman. Undaunted by their predictions of an energy gap he argues that "what we are really being offered is little more than predictions of a change from a brief period of energy self-sufficiency in the 1980s and 1990s to a renewed period of energy import dependence—mainly for liquid hydrocarbons—at a time when the real price of energy is likely to be significantly higher than it is today. The threat, if there is one, is therefore to the prospect of continued exponential growth of final energy demand . . . it is not, nor need ever be, a threat to a society with improving rates of efficiency in fuel conversion and energy use".

An energy forecast is impossible, says Forman, without making prior political assumptions; and one of those assumptions ought to be that demand can be modified to meet supply.

On reprocessing and the proposed facility at Windscale, Mr Forman argues that "there simply will not be the necessary nuclear fuel throughput

from the Continent or elsewhere to justify such a large investment". Reprocessing, in other words, is uneconomic. The same is true of the fast breeder, says the MP, pointing out that the construction of around 20 breeder reactors of 1,300 MW each would cost a total of some £30 billion. "Simply to postulate such an enormous figure is to underline the foolishness of committing this nation to such a single-minded and excessive expenditure on a form of energy supply which we are unlikely to be able to afford and may not even need".

It may shock them, but with Prime Minister Jim Callaghan bearing down on Energy Minister Tony Benn to make him take a more pro-nuclear line it is beginning to appear that the best thing the Friends of the Earth could do would be to vote Tory.

Robert Walgate

Soviets look after their cosmonauts

THE purpose of the Soyuz-Salyut programme is not the carrying out of separate experiments, however unique. In a recent Tass statement, Flight Director Vadim Kravets announced that the ultimate aim is the establishment of a permanent scientific watch in orbit.

Accordingly, much is being done towards cosmonaut comfort and better working conditions, including the installation of a shower in Salyut 6 and the introduction of new semi-rigid space suits for extra-vehicular activity.

Since Salyut 4, the special computer-based Kaskad orientation system has been used to re-align the station as required by the programme of geo-physical, astro-physical, and solar observations, while the Delta autonomous navigation system has eliminated the exhausting and tedious work of orbital correction.

The possibility of natural disaster in orbit is considered minimal—pilot-cosmonaut Georgii Grechko has been quoted as saying that a direct hit by a meteor would be likely only once in two thousand years. Nevertheless, a Tass report of 24 December notes that a micrometeorite groove 1.5 mm deep has been observed on the glass surface of one of the Salyut 6 portholes. Grechko remains unruffled. □

First catch your bear . . .

AN unscheduled contribution to Soviet-US co-operation in the ecology of the Far North has been initiated by an Alaskan polar bear which, wearing a collar with a radio-transmitter attached, has set out across the Bering Strait to Siberia. When last observed, the bear appeared to be making for Wrangel Island, an area in which unfortunately, no major expedition is working at present. It has been agreed, however, that should the staff at the reserve base on Wrangel Island spot the bear, the collar and its transmitter will be returned to the Americans. □

\$17m for Salk institute

THE Salk Institute for Biological Studies is to set up a new "government services division" to carry out contract research for various government agencies.

This move has been made possible by the donation to the institute of the vaccine research and production facilities of a major pharmaceutical manufacturer, Richardson-Merrill, Inc. The value of the facilities has been assessed at \$17m.

The manufacturing portion of the facilities has already been sold. The institute will retain the research portion of the facilities, situated in Swiftwater, Pennsylvania, including 25 professional and technical personnel. □

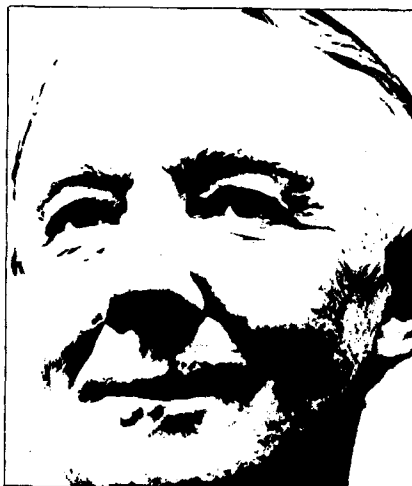
MAN, with his increasing numbers and his wasteful use of resources, may damage both his immediate environment and the globe as a whole. Most of us wish to try to make the world a better place to live in, or at least to prevent further damage to its natural amenities. However, those concerned have different approaches to environmental problems, and it is perhaps worthwhile trying to decide which is the most effective.

Most publicity is given by the media to the extreme 'doomwatchers', who think that the only way to produce results is to frighten mankind into mending his ways. This was the technique of the Victorian hell-fire preacher. Today some environmentalists seem almost to exult in the prospect of impending disaster, as did the earlier preacher at the probability that his listeners would suffer the tortures of eternal damnation.

These extremists are unwilling to agree that there has ever been any improvement—they may even believe that this is the case. They are scornful of those with a different approach, and have their own vocabulary of insults. Those who express even the most qualified optimism are "complacent", and when this optimistic picture is supported by evidence that at least one type of damage had been controlled this is described as "simplistic". Even when it is impossible to deny that there has been some improvement they think up cryptic and subtle dangers from which we are suffering without being aware of their presence.

On the other hand we have those who take a more moderate line. They know that resources are limited, and they wish to make sure that these are used effectively. They wish to identify and quantify all dangers, so as not to waste their efforts on trivial and unimportant factors. As this group tends

Postponing doomsday



KENNETH MELLANBY

to lack glamour, it is seldom seen on television.

One organisation which has a reputation for moderation is the National Society for Clean Air (NSCA). I attended their annual conference at Harrogate recently. The members of this society are mostly recruited from the local authorities which are responsible for environmental protection, together with government and other scientists expert in different fields. The atmosphere at their conference was one of hope, not of doom. Most of the papers recorded growing improvements in the air quality of urban and industrial areas in Britain, and few spectacular dangers of any magnitude were reported.

The relative unimportance of the existing levels of substances like carbon monoxide and lead, which would be disastrous at higher concentrations, was discussed. However,

the conditions in areas with laggard local authorities, where smoke control was not enforced, were fully described, and those responsible were duly castigated. The members of NSCA had no doubt that their own efforts had contributed substantially to the real improvements which they reported. Some listeners complained that the Society was unduly complacent, and that it should be more militant even when existing pollution levels were apparently harmless and were, anyhow, decreasing, but these views received little support.

It will be clear that my sympathies are with NSCA. They get things done. Extremists will say that this is, at least in part, because doomwatch publicity has affected public opinion, and made it ready to respond to moderate views which might otherwise be ignored. I am doubtful. In the late nineteen sixties we were told that growing air pollution would make the major cities of Europe and America uninhabitable before the end of the century. Most and most people can recognise that things have got better and not worse. The Victorian preacher did not stamp out sin; many of his congregation did not really believe in the hell he promised them. The doom-besotted environmentalist is in an even worse position, for there is concrete evidence that his particular hell, if it exists, is getting further away as the moderates get on with the job of environmental improvement.

However, the world is still far from perfect. The extremists are not always wrong, and though their efforts at frightening the general public may be counterproductive, the experts would be wise to pay at least some attention to their views, for the cryptic dangers they stress have, at least in some few cases, been shown to be real.

correspondence

In support of a boycott

SIR,—We read with great interest the letter by Richard Peto and Richard Doll (1 December, page 384) concerning the congress of the International Union Against Cancer which is scheduled to take place in Buenos Aires, Argentina October 1978 and the advisability of conducting a boycott in view of the violations of human rights in that country. It is stated that “advice and comments from other scientists, especially those who, unlike ourselves, have worked under repressive regimes, would be timely, both about the general advisability or inadvisability of such boycotts” in a country where there is conclusive evidence of widespread repression, torture and systematic violations of human rights.

The Association Solidarité Franco Argentine (a non-profit making, humanitarian organisation based in France) has contacted many Argentinian scientists regarding this problem. Although they asked not to be identified by name for fear of the consequences to their families still living in Argentina, the general opinion is that whatever measures are taken to show concern for the fate of the many scientists that have simply “disappeared” at the hands of the military government, there is one thing which should not be permitted: that the Argentinian government should use the success of the congress to claim to the world that the scientific community condones its present policies, by being present at the meeting and keeping silent about the plight of Argentinian scientists.

Unfortunately this is what seems to be happening in Argentina right now. A recent article from the strictly censored local press says that in spite of the efforts of some scientists, who pretend to be motivated for humanitarian reasons but in fact echo slogans of international terrorism, the congress will be a success. To prove this, the article mentions that only 500 participants had registered for the previous Florence meeting, compared to the 3,000 for the Buenos Aires event. Dr Humberto Veronessi, President of the International Union Against Cancer, is quoted as having said “I believe that to choose Buenos Aires for this meeting is one of the most

intelligent actions taken by the Union”. This type of article suggests that the Argentinian generals have already invested high hopes in a “business as usual” congress as a measure of international acquiescence.

Among the many well known scientists to have publicly protested against the terrorising policies of the military Junta are Nobel prize winners Louis Néel, Alfred Kastler, Tsung Dao Lee and Hans Bethe (*New York Times*, 27 November, 1977). Concerned scientists attending the Buenos Aires meeting can take similar action by openly voicing their concern about the many Argentinian scientists who have vanished. This could be done by organising a petition to be read at the meeting or a press conference to be held in the presence of foreign journalists.

What has happened to Drs Federico Alvarez Rojas, Antonio Missetich, Gabriela Carabeli, Eduardo Pasquini, Julia Huarque, Federico Ludden, Manuel Tarchitsky and Juan Carlos Gallardo, all of them physicists, and the many others kidnapped, in many cases, with their families? Why aren't the numerous concentration camps open to Red Cross personnel? There is every reason to suspect that the worst is happening, just as it did in Nazi Germany, where similar questions puzzled many scientists, and massacres were taking place not far from the place where scientific meetings were being held.

Exiled Argentinian scientists beg the rest of the scientific community to stand up, whether by boycotts or in other ways. “Anti-science” sentiments will certainly not be aggravated by a public stance against torture and death. Many lives may instead be spared.

Yours faithfully,

GUY TASSART

*Association Solidarité
Franco Argentine,
Grenoble, France*

Soft energy paths

SIR,—Peter Chapman is right to say (10 November, page 128) that my US calculations in *Soft Energy Paths: Toward a Durable Peace* should be redone for the UK—as several colleagues are now attempting. But in suggesting how my US numbers “fail on a num-

ber of technical issues” he may mislead UK readers unfamiliar with transatlantic differences.

First, he says my 0.55 capacity factor for US pressurised-water reactors (PWRs) is “unrealistically low”, “absurd”, and “silly”, and so it must seem to people used to British gas-cooled reactors which are far more reliable (though allegedly more costly). Yet my citations show that the US empirical average through 1976 is 0.61 (0.59 for all light-water reactors) and falling as less reliable larger plants come on line; that the officially projected lifetime average is 0.57; and that the 10-year levelised average expected for new big PWRs on the basis of exhaustive statistical analysis of existing units is 0.49. Thus my assumed 0.55 is arguable either way but hardly out of court under US conditions. (European units tend to do better, Japanese ones worse.)

Second, in my analogy suggesting that if we could mass-produce power stations the way we do cars they would cost an order of magnitude less than they do, but we can't because they're too big, I did take account of generator cost and of equivalent engine lifetime. (I also noted many important economies of small scale—related to reliability, reserve margin, short lead times, etc—which are not “very dubious” nor even empirically controversial. For a taxonomy of scale issues, see my “Soft Energy Technologies” in the 1978 *Annual Review of Energy*.)

Third, the \$100/(m²+m³) solar installed price Dr Chapman quotes is for the mid-1980s (in 1976 dollars), not now; my late-1970s estimate of \$150 is broadly consistent with his. My ca 1985 price estimate is based on empirical prices now obtainable by careful shopping in a fairly mature solar market (such as California, with due adjustments for different climatic needs). Current UK prices in an infant market with little volume or diversity are understandably higher but do not indicate what could be achieved. I do not see why the “cost of storage in the UK” should be £300/m³ or even £40/m³, as I cited empirical installed US prices, for modular underground tanks of tongue-and-groove concrete slabs, equivalent to £12-20/m³ for sizes of order 10–10² m³, and several even cheaper methods are available. Perhaps they are

not in use in the UK, but that is not my fault. Further, solar heat's marginal-cost advantage is not fragile but robust: even with collectors costing twice Dr Chapman's assumed £50/m², neighbourhood-scale seasonal-storage solar space heating in the UK should compete with any long-run marginal source and probably with OPEC oil too (see the *Ann. Rev. En.* article).

Fourth, my analysis assumed neither large wind machines nor growing special biomass crops (rather, it assumed the conversion of present farm and forestry residues requiring no additional land); and I did not ignore, but repeatedly emphasised, the economic argument for matching energy quality to end-use needs. One of the reasons for persistent official commitments to a hard energy path is the prevalence of asymmetric cost comparisons: governments compare the costs of various types of big power stations and synthetic-fuel plants with each other, then compare the costs of soft technologies not with their hard-technology competitors but with the historically cheap fossil fuels that all are meant to replace. This makes some soft technologies fail a test which hard ones would fail by a far wider margin. So long as such chicanery goes unremarked, economically and politically disastrous energy policies will continue to prevail over common sense.

Yours faithfully,

AMORY B. LOVINS

*Friends of the Earth,
London, UK*

of workers in communities where these experiments are undertaken, have not yet been implemented. And the body established to regulate work in this field (the Genetic Manipulation Advisory Group) has few powers, relies on voluntary cooperation and is already experiencing problems in dealing with confidentiality of industrial information.

The examination of this area by the select committee could achieve much. It could help bring about a much wider appreciation of the far-reaching issues involved. It could also provide a valuable independent assessment of the policy-making procedures being created in this area which are currently screened from the public gaze by the protection of the Official Secrets Act. Now is the time for such an examination, before the problems of industrial exploitation are upon us. This is an urgent matter which requires as thorough an analysis as that at last being given to nuclear power.

We applaud the initiative taken by the select committee and feel sure that it will take the opportunity to take note of the wide range of views on this topic.

Yours faithfully,

BRIAN CUMMINS

MARK PINEY

JON TURNER

NEIL WALDEN

EDWARD YOXEN

*British Society for Social
Responsibility in Science,
London*

Gene inquiry is timely

SIR,—Your editorial (8 December, page 461) criticised the decision of the House of Commons Select Committee on Science and Technology to set up a subcommittee on genetic engineering. It did so in remarkably complacent terms which seem to us to pass all too lightly over the problems which remain unexamined and unresolved in this area and to exaggerate the extent to which public debate has occurred. In our view it is not true to say there has been exhaustive scrutiny or debate in the UK of the issues involved. The "general feeling" of scientists involved in the field may be that the hazards have been overplayed, but this attitude ignores other issues which concern the public.

Many of the hazards involved in genetic engineering require much wider examination. The analysis so far has been far from comprehensive, and the actions taken incomplete. For example, recommendations made by the Ashby Committee three years ago, such as the institution of epidemiological surveys

What happened at Heimaey?

SIR,—In his search for a deontological code for volcanology, Haroun Tazieff (8 September, page 96) has elected not to practise his own preaching and indeed based some of his own arguments "on deliberately false data". We are astounded by his inclusion of the water-chilling of the Heimaey lava in 1973 in his tabulation of erroneous volcanological diagnoses, and his account of countermeasures taken by Icelanders as defence against lava flows on Heimaey indicate either lack of familiarity with relevant literature or wrong interpretation of actual facts.

In an attempt to prevent westward advance of the Heimaey lava over the town and towards the harbour, earth dams were bulldozed in late January and early February 1973. Experiments with chilling of lava-fronts by water-pumping started on 6 February and while lava advance could not be prevented, local slowing-down and diversion was achieved. Thus chilling of lava in this way is believed to have saved electric power-line installations for a while and diverted lava from the harbour wall on

6 March. Subsequently, pumps with total capacity of 1,000 litres per sec were employed, feeding a network of 20 cm diameter flexible plastic tubing system from the harbour to the lava fronts which were threatening further destruction of the town. This large-scale operation resulted in doubling of height of some lava fronts (Th. Einarsson, *The Heimaey Eruption*, Heimskringla, Reykjavik (1974)) as production of clinker and blocky rubble was increased on the lava surface. This increasing clinker accumulation rate seems to have decelerated or halted advance of the lava in certain areas.

Our knowledge of the mechanics of lava movement is still rudimentary. Recent theories, such as that of Hulme (*Geophys. Journ. Roy. Astr. Soc.* (1974)), make it clear that the strength of the flow front and channel levees are of great importance in controlling lava shape. As lava levees or flow fronts are made stronger and thicker, lava builds up behind these natural barriers. Lava will clearly attempt to break out or advance at the weakest front. By preferentially strengthening a levee or flow front by such a technique as water-cooling it seems probable that the lava will prefer to advance elsewhere. Levees or flow fronts are only minor parts of the total lava at any time, but increase in the strength of these areas may be highly effective in diverting lava. By choosing a strategic zone such as a levee, cooling need only be concentrated on a small part of the flow. Evidence from the Heimaey experiment suggests that the uncooled flow front ranged 10 m to 15 m in height, whereas the flow front treated by water pumping ranged from 20 m to 30 m and possibly as much as 40 m.

Finally there is a requirement to substantially improve our understanding of lava flow mechanisms. This is an area of research that illustrates the importance of volcanology turning from a qualitative to a quantitative science. In this way some of the noticeable subjectivity in judging volcanic phenomena, which is amply illustrated in Dr Tazieff's note, can be replaced by informed opinion, based on detailed understanding of the physics and chemistry of volcanic processes.

The determination of the people in Heimaey in fighting the advancing lava was not daunted by defeatist utterances of some sceptics at the time of the eruption. We hope that readers of the otherwise useful note by Haroun Tazieff will likewise dismiss his pessimism about the usefulness of water-cooling in diverting lava flows.

Yours faithfully,

HARALDUR SIGURDSSON

STEPHEN SPARKS

*University of Rhode Island,
Kingston, USA*

news and views

Tangshan 1976: a case history in earthquake prediction

from Cinna Lomnitz and Larissa Lomnitz

WAS the Tangshan earthquake of 1976, an event of Richter magnitude 7.8, predictable in the present state of earthquake forecasting in China? We shall attempt to show that it was not; in the absence of well-defined procedures of earthquake forecasting outside China our case rests on the detailed information obtained on two other large events, the Lungling and Yenyuen earthquakes, both accurately predicted by Chinese seismologists in 1976. A 10-day briefing in China has been of the greatest importance in making this report.

Chinese earthquake forecasting is a structured process of four stages: long-range, middle-range, short-range and immediate predictions. In the case of Tangshan a long-range prediction was issued in 1966, implicit in a directive of the Central Committee of the Communist Party, requesting seismologists to pay particular attention to North China and specifically to the Peking-Tientsin-Haicheng triangle. This directive was issued after the 1966 Hsingtai earthquake.

After the successful prediction of the Haicheng earthquake of 1975 it was generally expected that the long-term strains accumulated in the Peking-Tientsin area had been released (Fig. 1). This was a reasonable assumption because the region of North China is only moderately active. Yet there has been clustering of large events in time: for example, two shocks of magnitude 8 occurred in 1679 and 1695. No major earthquakes had previously been recorded in the immediate vicinity of Tangshan; the nearest events were a coastal shock ($M = 6$) in 1568, and an earthquake in Langcheng County ($M = 6\frac{1}{2}$) in 1624.

The earthquake occurred at 03:42:56 (Peking time) on 28 July, 1976. The location of the epicentre was 39.43°N 118.15°E , with a focal depth of 12 km. Twelve aftershocks exceeded mag-

nitude 6: the largest ($M = 7.1$) occurred on 28 July in Langcheng County—about 50 km to the northeast of the main shock.

Tangshan is in the centre of a rhomboidal area outlined by major crustal faults. The Tangshan fault proper is at least of Palaeozoic age; it is 40 km long, and runs along the top of a narrow uplifted block of Ordovician age. From geological and geophysical evidence it was concluded that the earthquake had been caused by a regional compressive stress from $\text{N } 86^\circ\text{E}$.

The main fault broke along an 8 km trace which went through the middle of the city of Tangshan. Right-lateral displacements of up to 1.58 m were observed. The vertical component of displacement of the east block was down towards the northern end of the fault break, and up towards the south. The trend of the fault trace was about $\text{N } 40^\circ\text{E}$; however, the fault trace of the main aftershock was nearly north-south. The displacement of this fault

was left-lateral. The aftershocks fell into two groups: the areal distribution and focal mechanisms for each group were consistent with each of the two fault traces. Hence the Tangshan earthquake may be described as a twin event.

The two earthquakes to be used as comparison were also twin events. The Lungling earthquakes (Southern Yunnan, 29 May, 1976; magnitudes 7.5 and 7.6) were successfully predicted; the immediate prediction was made by an amateur group at Lungling about 20 min before the first main shock. The Yenyuen earthquakes (Yunnan-Szechuan border, 7 November, 1976; magnitude 6.9, and 13 December, 1976; magnitude 6.8) were also predicted but the immediate forecasts were issued 3 days in advance. No lives were lost in any of these quakes.

Forerunners

At least 10 different kinds of phenomenon are monitored for purposes of earthquake prediction in China. They include observations of fault creep, regional and local tilt, gravity, shallow earth strains, direct current resistivity, groundwater fluctuations and changes in spring flow, telluric currents, geomagnetic observations, geochemical observations (particularly of radon in groundwater), changes in the velocity of seismic waves, changes in the distribution of small earthquakes in space, magnitude or time and observations of foreshocks. All these are quantitative forerunners. There are also a few qualitative forerunners, such as changes in animal behaviour and in the groundwater regime: these are termed 'macro-forerunners'.

The following forerunners were identified in connection with the Tangshan earthquake.

- Fault creep. Two stations located at 180 and 200 km from the epicentre, on the Babaashan Fault, recorded a 25-fold increase in the creep rate after May 1975. The fault became locked

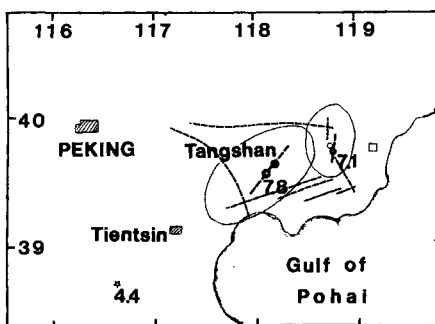


Fig. 1 Map of North China indicating the epicentre of the Tangshan earthquake (Peking magnitude = 7.8), and the second main event near Langcheng ($M = 7.1$). The ellipses represent the aftershock regions. The epicentre of the 'warning shock' near Tacheng ($M = 4.4$) is shown near the south edge of the map. Crustal faults outlining the 'Tangshan Quadrilateral' are indicated by broken lines; the two faults associated with the major events are represented by thick lines. The square symbol indicates Changli, a station which recorded a resistivity anomaly on 23 July, 1976.

between October 1975 and April 1976, then accelerated again to 33 times the average creep rate. About 20 days before the earthquake the direction of fault motion was suddenly reversed—unfortunately, at a time of heavy rainfall. (The effect of rainfall on the motion of the Babaashan Fault is not fully understood, but it is known to be a major effect).

● Gravity. Between July 1974 and July 1976 there was an increase of $100 \mu\text{gal}$ in the Bouguer anomaly over the Tangshan quadrilateral. This was considered to be barely significant because the mean error was estimated at $40 \mu\text{gal}$.

● Strain measurements. Between May 1975 and July 1976 the quartz-rod strainmeter at Dahuidyan Observatory, across the Babaashan Fault (180 km from the epicentre), recorded a shortening of 0.3 mm, indicating regional compression. Piezoelectric strainmeters buried in pipes at depths of 3–10 m detected a sudden strain anomaly immediately before the earthquake. Dilatations and compressions were distributed in quadrant fashion about the epicentre.

● Direct current resistivity. Changli station, 80 km east of Tangshan, recorded a large decrease in resistivity beginning on 23 July. Between 30% to 40% of resistivity stations in North China showed some anomaly, but none was as clear as the one at Changli. The anomalies were widely scattered over distances of up to 300 km from the epicentre.

● Fluctuations in wells. Taking 23 July as a pivotal date, there was a marked increase in the reports of anomalies in wells. Only 59 such reports came in during the 5-day period preceding 23 July, but the number increased to 665 in the following 5 days.

● Telluric currents. Measurements of telluric current were not found to be particularly useful as the records were erratic. At Hsingchuang station, near Tientsin, a large telluric peak was recorded 13 min after the main shock, possibly due to some seismic coupling effect.

● Geomagnetism. No geomagnetic anomalies were mentioned at any time.

● Radon. Most Rd values had been increasing in North China since 1968. After February 1974 there were seemingly erratic fluctuations; after the Tangshan earthquake the Rd content has been dropping nearly everywhere. But there were no specific anomalies in the Tangshan area.

● Small-scale seismicity. There was no clear decrease in the minor seismicity after the 1975 Haicheng earthquake, as might have been expected if the regional strains had been released. A seismic gap had developed in the 'Tangshan quadrilateral' since about

Table 1 Short-range to immediate prediction

Time	Tangshan	Lungling	Yenyuen
$E, 9 \text{ days}$	Resistivity drops at Changli Station	Increasing anomalies of Rd, magnetics, wells, hot springs, others	First foreshock, $M = 4.3$. Tsuli well dries up.
$E, 3 \text{ days}$	Increase in well reports	Tsuli well dries up	Short-range forecast: $E, 2 \text{ d}, M = 6-6.5$
$E, 2 \text{ days}$			Second foreshock, $M = 4.0$. Tsuli well recovers
$E, 1 \text{ day}$		Short-range forecast: $E, 2 \text{ d}, M = 5-6$	Immediate forecast: Evacuation
$E, 10 \text{ hour}$		Tzushun tilt base shows anomaly	Tzushun tilt base shows anomaly
$E, 25 \text{ min}$		Large magnetic anomaly; animal behaviour; wells	
$E, 0 \text{ Earthquake}$		Foreshock, $M = 5.2$	
		More foreshocks	
		Immediate forecast: Evacuation	

1967; during 1975 this gap was outlined more clearly as only 14 shocks of magnitude 2.5 or greater occurred within the gap. The minor seismicity tended to migrate towards Tangshan: by 1976 many small shocks were occurring close to the edge of the gap.

Within the Tangshan quadrilateral the b -value decreased from 1.0 to about 0.35 between 1967 to 22 April, 1976. On this date an earthquake of magnitude 4.4 occurred in Tacheng (Hopei), near the southern edge of the gap. Afterwards the b -value recovered to about 0.6 just before the Tangshan earthquake.

The v_p/v_s anomaly about Tangshan followed a similar pattern. However, these anomalies were apparently detected after the earthquake, not before.

● Foreshocks. The seismographic record of the Tangshan station was recovered from the ruins: it showed only a few local shocks of magnitude less than 2 during the hours preceding the earthquake. This is conclusive evidence of the absence of foreshocks.

A comparative time history of forerunners of the Tangshan, Lungling and Yenyuen earthquake is shown in Fig. 2. At first sight no significant difference in the patterns emerges—up to the intermediate-range prediction. An intermediate-range prediction was indeed made for all three events. The magnitudes of all three earthquakes were underestimated. The differences appear at the short-range prediction stage. The comparative table above clarifies this point (E =time before the earthquake).

Notice that geophysical reversals (marked with an arrow in Fig. 2) were conspicuously absent before the Tangshan earthquake. The two exceptions (b -values and fault creep on the Babaashan Fault) were detected after

the earthquake, and neither could have been used to pinpoint the epicentral area. The Tacheng 'warning shock' of magnitude 4.4 occurred at a considerable distance from Tangshan. In contrast, foreshocks did occur both at Lungling and at Yenyuen, thus enabling local seismologists to close in on the epicentre weeks in advance of the earthquake. The onset of major foreshock activity, combined with sudden reversals of local tilt, magnetic field or fluctuations in ground water, will usually trigger an immediate prediction followed by evacuation.

A few words about the philosophy of earthquake prediction in China may be in order. The 1966 directive, usually attributed to Chou En-lai, outlined a five-point policy of earthquake prediction research: (1) give priority to prevention; (2) join the efforts of specialists and amateur workers; (3) combine indigenous and foreign methods; (4) work through the masses, (5) under the leadership of the Party. This policy has led to a distinctive scientific approach to seismological work. Initially many Chinese seismologists were reluctant to abandon deductive procedures based on the concatenation of hypotheses rigorously tested under controlled field situations. The key role of the masses was not recognised. Yet the observatories manned by specialists were too far-flung to be of practical use in detecting forerunners. Thousands of 'mass stations', built and operated by amateur observers, were needed to fill the large gaps between professional stations. Even those Chinese colleagues who remain moderately sceptical about earthquake forecasting methods now readily concede that the close collaboration between professional scientists and amateur observers represents a key element in achieving successful predictions.

Chinese seismologists do not claim any special knowledge concerning the cause and mechanism of earthquakes. After the successful prediction of the 1975 Haicheng earthquake, an official of the State Seismological Bureau bluntly warned: "There will be errors and disappointments, as well as unavoidable mistakes in reporting or failing to report individual forecasts . . . Earthquake prediction is a major complex scientific problem which overlaps many disciplines and which can hardly be expected to be solved at short notice . . . We have a long way to go. It will take time and much hard work". This note of caution, sounded 7 months before Tangshan, was by no means an accidental or isolated statement. Serious failures of prediction have occurred before the Tangshan disaster and will occur again. However, as long as the mechanism of earthquake generation is as imperfectly understood as it is today, the Chinese approach to prediction represents probably the most effective tool for controlling earthquake hazards in seismically active regions.

We are grateful to the State Seismological Bureau in Peking and to Chinese seismologists, for permission to reproduce the results of their investigations. Special thanks are due to Gao Wen-hsue of the State Seismological Bureau, Jin Hsue-shen and Li Yu-so of the Hopei Province Seismological Bureau, Ko Shen-min of the Institute of Geology, Ku Kung-hsu, Fu Cheng-yi, Sung Liang-yu, Chu Chun-zhen and

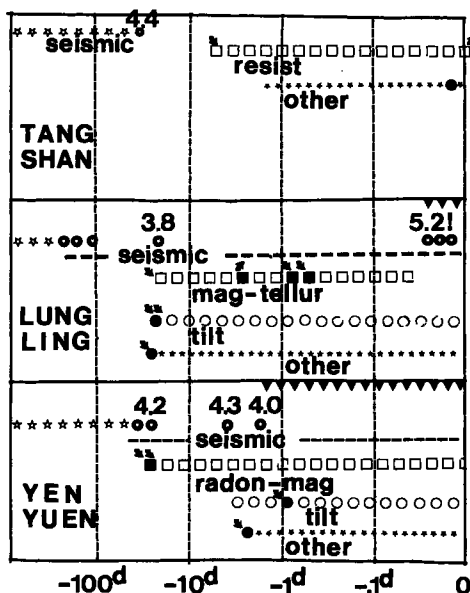


Fig. 2 Forerunners of the Tangshan, Lungling and Yenyuen earthquakes, as referred to the time of the first main shock. A sawtooth pattern at the top indicates evacuation following an immediate prediction. Note that foreshocks serve to pinpoint an epicentral area, which is helpful in interpreting other anomalies. Intermediate prediction in all three cases occurred 15–150 days before the shock. Arrows, reversals; filled symbols, anomalies detected as significant.

Shi Jen-liang of the Institute of Geophysics, and Sung Chang-jiang, Chu Cheng-nan, Wang Feng-chi and Lin Tsun-yun of the Yunnan Province Seismological Bureau. □

Reabsorption of digestive enzymes: playing with poison

from Jared M. Diamond

In the course of digestion, the intestine and associated glands secrete quantities of solutes and water so large that the body could ill afford to lose such quantities in the faeces. Hence it is no surprise that distal parts of the intestine reabsorb most of these substances. Physiologists have studied in detail how, in a cycle termed the enterohepatic circulation, bile salts secreted by the liver are reabsorbed by the ileum and return by way of the bloodstream to the liver; and how salts and water secreted by salivary glands, stomach, liver, pancreas, and intestinal glands are reabsorbed between the duodenum and colon. Nevertheless, until recently there was no awareness of a cor-

responding circulation of the enzymes which the pancreas secretes into the intestine to split proteins, fats, and starches. Indeed, when evidence for such an enteropancreatic circulation was reported in 1975, it aroused 'interest but alarm' (on the part of Beynon and Kay, *Nature* 260, 78; 1976).

Reasons for the alarm were several. First, enteropancreatic circulation would require protein absorption by the intestine. While this has been demonstrated in some studies, much more attention has been paid to protein hydrolysis to dipeptides and amino acids in the gut lumen and subsequent absorption of the dipeptides and amino acids. Second, proteolytic enzymes and lipases might be expected to wreak havoc in the bloodstream, as illustrated

by their potency as the active ingredients of many venoms. Finally, there was no evidence for protein uptake from the bloodstream by pancreatic cells.

The missing evidence, and cause of the alarm, was provided by Rothman and colleagues (University of California Medical Center, San Francisco), who have added to the evidence (and to the alarm) in recent publications (*Science* 189, 472; 1975; *Nature* 257, 607; 1975; *Lancet*, 4 September, 494; 1976; *A. Rev. Physiol.* 39, 373; 1977; *Proc. natn. Acad. Sci. U.S.A.* 74, 4068; 1977). Their chain of evidence is as follows.

- Fifteen minutes after instillation into the gut of radioactive chymotrypsinogen, the inactive form of the proteolytic enzyme chymotrypsin, the radio-label appears in the pancreatic juice in a form comigrating electrophoretically with chymotrypsinogen.

- If pancreatic juice is diverted from entering the gut lumen and is instead drained off by a surgical fistula, protein secretion by the pancreas decreases 80–90%. Protein secretion is restored if pancreatic juice is returned to the gut or bloodstream. The calculated efficiency of enzyme recirculation, 80–90%, is comparable to that of the enterohepatic circulation of bile salts.

- The starch-splitting enzyme amylase is absorbed with its activity intact by a mechanism dependent on aerobic metabolism in the ileum. Absorption of a control high-molecular-weight substance, the physiologically inert polysaccharide inulin, is negligible under the same conditions. Similarly, Papp, Feher, Folly, and Horvath (Hungarian Academy of Sciences, Budapest) have reported duodenal absorption of pancreatic lipase intact (*Abstract, 1977 European Pancreatic Club Meeting*, 23).

- Radiolabelled chymotrypsinogen and amylase in the solution bathing or perfusing the isolated pancreas are taken up by the pancreatic cells and appear in pancreatic juice. The secretion rate of radiolabelled amylase in juice is increased by pancreozymin, the hormone that stimulates amylase secretion physiologically. That these phenomena are not due to breakdown and reincorporation of the radiolabel is shown by the negligible label in juice if the radiolabelled protein presented is albumin; by the absence of label in secreted amylase if the label presented is chymotrypsinogen; and by inhibition of labelled chymotrypsinogen secretion by an excess of unlabelled chymotrypsinogen, as expected for a saturable transport mechanism.

- There is a significant flux of amylase from pancreatic cells to an external bathing solution as well as into the pancreatic duct. Controls argue against

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this being attributable to a duct-to-bath shunt or cell damage. Thus, there is a two-way flux of amylase across the pancreatic cell membrane facing the bloodstream.

● Instillation of greater than normal quantities of pancreatic juice into the intestine of rabbits leads to 50% mortality—the only plausible toxic agents in pancreatic juice being the pancreatic enzymes themselves.

Mysteries remain. For efficient use of the enzymes in the gut, intestinal absorption should occur distally in the ileum as for bile salts, yet Papp *et al.* reported proximal (duodenal) absorption. Injection of proteases and lipases into a victim's bloodstream by poisonous snakes has spectacular consequences; why are the consequences of the postulated enteropancreatic circulation not equally spectacular? Are only the inactive forms of the enzymes absorbed by the intestine? If so, are the active forms lost in the faeces, and how can this be reconciled with 80–90% efficiency of recirculation? Does the intestine itself inactivate the enzymes while absorbing them? Both alarm and interest may be expected from physiologists until these questions are answered. □

Liposomes in biology and medicine

from G. Gregoriadis

A conference on Liposomes and their Uses in Biology and Medicine was held under the auspices of the NY Academy of Sciences on 14–16 September, 1977. It was chaired by D. Papahadjopoulos, Roswell Park Memorial Institute, Buffalo.

LIPOSOMES have been developed over the past 10 years or so both as model systems for the study of cell membranes and as possible vehicles for directing drugs to specific targets in the body. These two apparently unrelated aspects of the liposome were brought together at the conference.

The plethora of data on the physical properties of liposomes in their role as model membranes is now proving essential to the study of their interaction with cell membranes in their role as drug delivery vehicles. The opening lectures therefore—on the molecular organisation, lipid mobility, thermotropic and other properties of liposomes—were extremely relevant to their therapeutic applications. Fluidity

of liposomal membranes, for instance, which determines many of their structural and biological functions (interaction with cells for example) can be modulated by the incorporation of various molecules, by the hydrogenation of unsaturated phospholipid components (D. Chapman, University of London) or by Ca^{2+} which induces phase separation and subsequent fusion between liposomes (D. Papahadjopoulos, Roswell Park Memorial Institute, Buffalo).

Evidence of endocytosis as a mode of liposome–cell interaction was supplemented by alternative mechanisms such as fusion with mammalian cells (G. Poste, Roswell Park Memorial Institute), bacteria (M. J. Osborn, University of Connecticut Health Center) or viruses (A. M. Haywood, University of Rochester), as well as adsorption (R. E. Pagano, Carnegie Institution of Washington, Baltimore). However, such mechanisms, which relate to liposomal membrane fluidity and to cell or viral membrane components, may be less relevant *in vivo*. For instance, fusion has been seen in the absence of serum, components of which (α_2 -macroglobulin for example) adhering to liposomes could promote phagocytosis. Nonetheless, the possibility that mechanisms could be selectively induced to enable the access of liposomal drugs to a particular target organelle was raised by C. de Duve (International Institute of Cellular and Molecular Pathology, Brussels) who in this way introduced the conference to the exciting prospects of medical applications. These ranged from the treatment of tumours, enzyme and hormone deficiencies, metal storage diseases and arthritis to immunopotential and interferon induction.

Cancer chemotherapy, which perhaps unjustifiably dominated the meeting, covered conditions (such as lipid composition) under which liposomal drugs (for example, cytosine arabinoside (E. Mayhew, Roswell Park Memorial Institute; T. Kataoka, Japan Foundation of Cancer Research, Tokyo) or methotrexate, (H. K. Kimelberg, Albany Medical College)) given to ascites tumour-bearing mice prolonged survival more than did the free drugs. Although reduction of toxicity to normal tissues was a possible reason (Y. E. Rahman, Argonne National Laboratories) others, such as slow sustained formation of a more active metabolite (Ara-C triphosphate, Buffalo group) or sustained release of the drug itself were also thought likely. In connection with the possible treatment of lung metastases, markedly retarded removal of drugs from the lungs of animals given liposomal drugs by way of the airways was reported by R. L. Juliano (Hospital for Sick

Children, Toronto). Furthermore, localisation in animal tumours of a liposomal marker given intravenously was seen after scanning (B. E. Ryman, Charing Cross Hospital, London).

Ways to smuggle poliovirus into virus-resistant cells (R. Taber, Roswell Park Memorial Institute) and of enzymes into enzyme-deficient cells (G. Weissmann, New York University Medical Center) with the help of liposomes were of great interest and uptake of enzymes could be directed into specific cellular regions by appropriately designed liposomes. *In vivo* however, selectivity is much more difficult to achieve, especially in view of hepatic and splenic involvement. Some of the many properties of liposomes which influence their redirection to alternative sites as well as the role of the 'travelled' biological space (blood for example) were discussed (G. Gregoriadis, Clinical Research Centre, Harrow). Small liposomes could localise in target (tumour) areas more efficiently and liposomal lipid composition is essential in promoting the entrance of insulin given intragastrically into the periphery or in producing a better immune response to diphtheria toxoid. Homing of liposomes to specific cells, previously shown to occur *in vitro* by way of anti-target cell immunoglobulins incorporated into the liposomal surface, and successfully applied to the selective stimulation of lymphocytes (W. E. Magee, University of Texas, San Antonio) was, however, marred *in vivo* by the excessive participation of the liver.

In spite of their almost unlimited potential, liposomes have scarcely been investigated in man and the only therapeutic attempts have been enzyme replacement therapy of lysosomal storage diseases. An infant with glycogenosis type II was given liposome-entrapped fungal amyloglucosidase but died before long-term observations could be made (Ryman). In a case of adult Gaucher's disease (glycosphingolipidosis) afflicting the reticuloendothelial system (liposomes home there spontaneously) the patient was repeatedly given liposomal human glucocerebrosidase: β -glucosidase over an 18-month period. There was marked relief of pressure and associated pain in the lower abdomen presumably as a result of a reduction in liver size. Each treatment was followed by pain in the liver which was proportional to the amount of enzyme used but which was absent with 'empty' liposomes (Gregoriadis). The ethics of the use of liposomes in such clinical trials before extensive animal experimentation were questioned. However, in view of the routine use of (Intralipid) phospholipid in intravenous feeding, selected phospholipids are likely to be less toxic

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than many of the cytotoxic drugs already in use, and it is rather the unknown way in which liposomes mediate drug action (or even toxicity) that one should consider. The implications are that under appropriate conditions, studies on marker-bearing liposome distribution in man could be a harmless and informative exercise, and that liposomal toxicity should always be examined in conjunction with the administered drug. Even so, in the absence of suitable animal models, the use of liposomal therapeutic agents in man might be acceptable. This is the case, for instance, with some human enzymes which in their free form have not been associated with toxicity and have not shown any beneficial effect.

The general feeling at the end of the conference was that although liposomes were already established as tools in the study of cell behaviour, their successful application in medicine was still a matter of speculation. On the other hand, as conventional drug treatment has in many instances proved unsuccessful, controlled drug delivery appears to be a hopeful alternative. In this respect, it is anticipated that liposomes will play a prominent part. □

A new human hepatitis virus

from Arie J. Zuckerman

THE identification of specific viral antigenic markers of hepatitis A (infectious or epidemic hepatitis) and hepatitis B (serum hepatitis) during the past few years has enabled sensitive laboratory tests to be developed. These, in turn, have led to a better understanding of the epidemiology, pathogenesis, immunology and nature of these infections; and, in the case of hepatitis B established the significance of this virus in severe chronic liver disease including at the very least a strong association with primary hepatocellular carcinoma in tropical and some subtropical regions. In addition, the specific diagnosis of hepatitis type A and hepatitis type B has revealed a previously unrecognised form of hepatitis which is clearly unrelated to either type. It is now the most common form of hepatitis occurring after blood transfusion in some

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Polymer diffusion disentangled

from P. D. Calvert

IN 1971 de Gennes predicted that the diffusion coefficient of a polymer chain in a gel would be proportional to the inverse square of the molecular weight (M) and with some reservations extended this prediction to self diffusion of molten polymers (*J. Chem. Phys.* **55**, 572; 1972). At the time the only available data, from NMR measurements by McCall *et al.* (*J. Chem. Phys.* **30**, 771; 1959), gave an exponent of $-5/3$. In this issue of *Nature* (page 143) Klein presents data for the self diffusion of polyethylene which confirms de Gennes prediction.

Self diffusion is not really an important property of polymers and most diffusion measurements are for gases or other small molecules; previous work of Klein and Briscoe and others in this field was described in a recent *News and Views* (**269**, 290; 1977). Viscosity is, on the other hand, an important property since most plastics are formed by squirting liquid polymer through small holes. Both self diffusion and viscosity involve molecules moving relative to one another, so one usually expects the diffusion coefficient to be inversely proportional to the viscosity.

Diffusion and viscosity in dilute polymer solutions has been well explained using Rouse models, where the polymer chains are viewed as strings of beads joined by springs and acted on by the flowing solvent. Molten short chain polymers can be treated similarly and one expects and gets a viscosity which increases proportionally to molecular weight, that is to chain length. At a critical molecular weight characteristic of each polymer this tidy state of affairs stops, and melt viscosity starts increasing proportionally to molecular weight to the power of 3.4. The reason for this is that the chains become entangled and difficult to separate. Exactly how this works and why the exponent is 3.4 has never been properly explained, though people have managed to obtain the right answer by rather

unconvincing routes.

De Gennes approached this problem by considering a chain molecule moving through a cross-linked network, so that the moving chain must wriggle through the network in a snake-like fashion. De Gennes christened this motion reptation. In essence the network chains can be thought of as forming an elastic tube within which the free molecule is constrained to move. This idea can be extended to molten polymers if we allow that the tube itself can move and reform, so that chain motion will be a combination of motion within the tube and motion of the tube. Reptation, motion within the tube, gives diffusion dependent on M^{-2} and viscosity on M^3 (de Gennes *Macromolecules* **9**, 594; 1976). Motion of the whole tube was tackled by Edwards and Grant (*J. Phys.* **A-6**, 1169, 1186; 1973) who showed that this gave a diffusion dependence of M^{-3} and viscosity of M^3 , and thought that this motion would dominate in molten polymers. What we conclude from Klein's results is that the tubes do not seem to move and reptation dominates, at least in diffusion. However, this still leaves us with the mysterious $M^{3.4}$ dependence observed for viscosity.

The idea of entanglements arose when workers on rubber elasticity found that cross-linked polymers acted as if they contained more junction points than are actually put in. It is a convenient concept, but carries with it the notion that a few interactions between chains act as tie points which are important for their properties, whilst the vast mass of interactions have little effect. It seems that reptation may lead to entanglements becoming redundant. Their replacement with a more realistic description of friction between random chains would be a real improvement.

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areas of the world (*Report of the WHO Expert Committee on Viral Hepatitis*, Techn. Rep. Series, No. 602, 1977). Although there are as yet no specific laboratory tests for identifying this new form of hepatitis, recent studies nevertheless stress the importance of this infection.

Results obtained from several recent surveys of post-transfusion hepatitis in

the United States provide strong epidemiological evidence of 'guilt by association' of an infection of the liver referred to in the USA as non-A: non-B hepatitis. Perhaps the most convincing report has just been published by Hoofnagle and colleagues (*Ann. intern. Med.* **87**, 14; 1977) after retesting serum samples that had been frozen from transmission studies con-

ducted in volunteer male prisoners in the USA in 1951-54 as part of a study on means of inactivating hepatitis virus in blood. The viral aetiology of hepatitis was in fact established by successful transmission experiments to volunteers first in Germany in 1942, in Palestine in 1943 and later by more extensive studies carried out in Britain and in the United States. Because a wide range of laboratory and other animals including monkeys, pigs, gerbils, jerboas, desert rodents and others had been inoculated with negative results permission for human inoculation tests was sought and obtained (*History of the Second World War. United Kingdom Medical Series. Medicine and Pathology*, 252 (Eds MacNalty, A. S. & Zachary Cope, V.) HMSO, 1952). In the series of volunteer studies carried out in the USA in the early 1950s, serum samples from six blood donors, implicated in the transmission of post-transfusion hepatitis after a single unit transfusion, were each inoculated into groups of 10-20 male prisoners. Sera from five of the implicated donors induced hepatitis in recipients. The serum samples collected during these studies have now been tested by Hoofnagle *et al.* for evidence of infection with hepatitis virus type A and B, cytomegalovirus and EB virus. Two of the donor sera contained markers of hepatitis B virus and transmitted hepatitis B infection to all susceptible recipients, two of

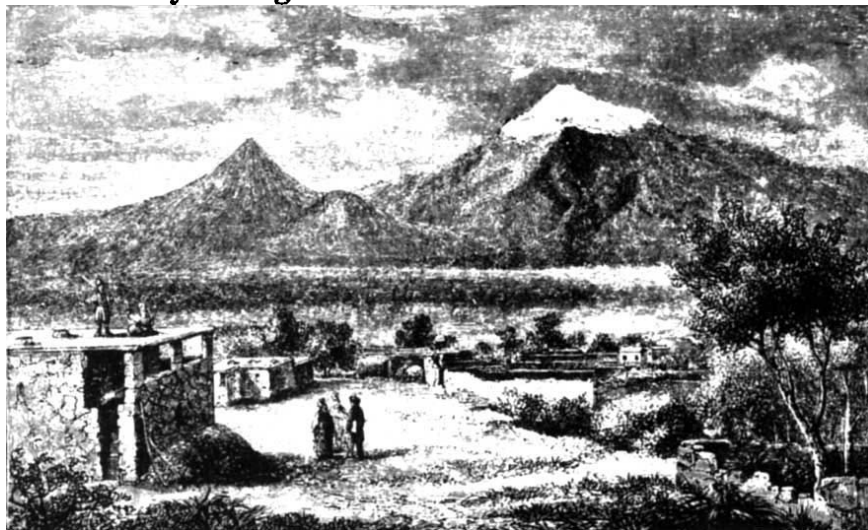
whom developed hepatic coma. The sera from the remaining three donors did not contain hepatitis B markers, but these sera were nevertheless infectious. A form of hepatitis was transmitted to nine volunteer recipients which could not be attributed to hepatitis A, hepatitis B nor to infection with cytomegalovirus or EB virus. The incubation period ranged from 18-89 d and the clinical illness was similar in most respects to hepatitis B. Of added significance was the finding of some evidence of persistent liver damage in one out of ten recipients who developed hepatitis type B and in two out of six volunteers who developed the unspecified type of hepatitis (non-A: non-B hepatitis).

In a recently completed survey on the efficacy of immunoglobulin (gamma globulin) for the prevention of post-transfusion hepatitis, Knodell and colleagues (*Gastroenterology* 72, 902; 1977) identified 44 patients with acute non-A: non-B hepatitis. Sixteen of the patients had persistent biochemical evidence of liver damage 6 months after the acute illness. In 10 patients the abnormality of liver enzymes continued for 1-3 years and liver biopsy in each patient revealed histological evidence of chronic liver damage. It was further observed that 9 out of 22 patients who developed evidence of chronic hepatitis received albumin solution as a placebo, whereas only 1 of 11 patients who were given

normal immunoglobulin developed chronic hepatitis and none of 11 patients who received hepatitis B immunoglobulin. Further evidence for the existence of a third type of human viral hepatitis is provided by Dienstag and colleagues (*Ann. intern. Med.* 87, 1; 1977) who investigated the cause of acute hepatitis in 45 patients admitted to a hospital in Los Angeles. There was no laboratory evidence of infection with hepatitis A, hepatitis B, cytomegalovirus or EB virus in 20 patients. It was noted that in nearly half of these patients infection followed the type of exposure traditionally associated with hepatitis B such as blood transfusion, plasmapheresis and occupational contact with patients.

These reports suggest the following: first, a carrier state of this newly recognised hepatitis agent since the infection is transmissible by blood transfusion (and probably by other routes) from apparently healthy donors; second, the infection must be common since pooled immunoglobulin seems to contain antibody as shown by the prophylactic efficacy of immunoglobulin in one study, and finally it seems that this infection may progress to chronic liver disease. The aetiological spectrum of viral hepatitis in man has thus been dramatically widened and there is an urgent need to develop specific laboratory tests for identifying this previously unrecognised virus or viruses. □

A hundred years ago



Great and Lesser Ararat from the North-east

So impressive a mountain, so long associated with man's faith and history, would have been appropriately placed among the most ancient landscapes of the earth's surface. Some scenes suggest only the changes of yesterday; others set us thinking of the earliest condition of our world. We naturally look for a kind of consonance between the venerable antiquity of the associations

which gather round Ararat and the primeval character of the finds, on closer research, that while most of these ridges have received their latest upheavals at a recent geological date, they yet for the greater part belong originally to earlier periods of disturbance, some of them, indeed, bearing witness to many successive uplifts during a vast section of geological time.

Yet further examination will bring before him evidence that along some of these lines of earth-folding, volcanic action has of old been abundant; and that the present Mediterranean volcanoes are but the lingering remnants of the chain of actively burning mountains which ran through Asia Minor and crowned the peaks of the Caucasus. And he will discover that just as there have been successive uplifts of the same axis or mountain-chain, so have there been widely-separated outbursts of volcanic activity during a long course of ages from the same focus of discharge.

In the middle distance is shown the alluvial plain of the Araxes. Below the snowy cone and icy cliffs of the Greater Ararat a deep cleft or recess appears with huge cliffs somewhat like the Val del Bove of Etna, and no doubt due to some of the volcanic explosions of the mountain. On the sky-line of this slope, towards the base of the larger cone, some of the late cinder-cones and craters appear. Some of these are still so fresh and perfect that they look as if they had been active only the other day and might blaze forth again tomorrow. The graceful outline of the Lesser Ararat rises on the left.

From *Nature* 17, 10 January, 205; 1878.

review article

Chromatin

Gary Felsenfeld*

The approximate shape of the chromatin subunit called the nucleosome is now known, but its internal architecture is not well understood. Recent studies reveal details of the organisation of DNA within the nucleosome, and show that the arginine-rich histones are essential to DNA folding. Nucleosomes or structures related to them seem to be present at points of DNA replication and transcription; interactions within and between nucleosomes are likely to play a critical part in these processes.

RECENT studies of chromatin structure have focused on the nucleoprotein subunit called the nucleosome¹⁻⁹. Starting with this one certain structural element, investigators have worked in two directions, either examining the internal structure of the nucleosome, or speculating on ways of assembling arrays of nucleosomes to give the higher orders of structure and DNA compaction that are characteristic of chromatin in the nucleus.

The canonical nucleosome consists of a well defined length of DNA^{7,8} (about 200 base pairs) complexed with an octamer³ of histones. The octamer contains two copies each of the slightly lysine-rich histones H2A and H2B, and the arginine-rich histones H3 and H4; H1 is not part of the nucleosome, but is associated with it. As new data have accumulated, these general features of the nucleosome model have been confirmed, but the details have, not unexpectedly, been modified. For example, it is now clear that the amount of DNA per histone octamer can vary between about 140 and 240 base pairs, depending upon the organism and tissue from which the nucleosomes are isolated¹⁰⁻¹⁶; even within a single cell type, the spacing is not homogeneous^{15,17-19}. The evidence for this variability comes from studies in which nuclei are digested briefly with staphylococcal nuclease. The first points of attack are within the sensitive DNA 'spacer' or 'linker' regions separating the repeating units. This limited digestion releases nucleosome oligomers and monomers that are close to full-sized, and extrapolation to zero digestion time gives the size of the fundamental repeat. It is the repeat measured in this way that is found to vary with the source of the nuclei. Further digestion results in rapid preferential degradation of spacer DNA, leading, in the case of the monomer, to formation of a relatively stable nucleosome 'core' containing the histone octamer and a DNA segment 140 base pairs in length²⁰⁻²². The size of this segment is invariant in all chromatin samples so far examined^{10,12,15}. The variability of the length of DNA in the nucleosome repeat thus derives entirely from variation in the length of the spacer regions that separate the cores.

The earliest physical studies of nucleosome core structure suggested a particle roughly spherical in shape, about 100 Å in diameter. There was also a proposal, however, that the nucleosome was disk-shaped²³. Neutron scattering studies, which measured the contribution of the DNA to the radius of gyration of the particle, unambiguously placed the DNA at the exterior of the nucleosome^{6,24,25}. Recently, detailed study of the neutron and X-ray scattering behaviour of nucleosomes in solution has

led investigators at Searle²⁶⁻²⁸ to conclude that the nucleosome could not be spherical. Their data were fitted best by a flattened cylindrical structure about 100 Å in diameter and 50 Å in height, with the DNA wrapped around it to form a pair of rings at the top and bottom.

Though solution scattering studies can eliminate possible models of the core shape, they cannot be used to prove them unambiguously. Fortunately, more direct information about nucleosome core shape has now been provided by the work of Finch, Klug, and their collaborators²⁹, who have crystallised nucleosome core preparations and carried out combined X-ray diffraction and electron microscopic measurements with a resolution of 20 to 25 Å. Some caution is necessary in comparing their results with those from neutron scattering, since the crystallised core particles contained histones that had undergone partial proteolysis. However, there is reason to think that this results in only minor changes in structure. The crystallographic data show that the nucleosome core is a flat disk about 110 Å in diameter and 57 Å in height, in excellent agreement with the neutron scattering model. Given the present resolution, the exact path of the DNA cannot be determined, though the electron density map is not inconsistent with the location of DNA on the periphery of the particle, as demonstrated earlier by neutron scattering. If it is assumed that the 140 base pairs of DNA form a uniform superhelix wrapped around the cylinder, the superhelix must have a diameter of about 90 Å and a pitch of about 28 Å, corresponding to between 75 and 82 base pairs per superhelical turn of B-form DNA.

Action of nucleases on the nucleosome core

Additional inferences²⁹ about the structure can be made by combining information from diffraction with data obtained from nuclease digestion studies. It has been known for some time that the DNA of the nucleosome is susceptible to internal cleavage by nucleases. Although the spacer DNA is the preferred target, the nucleosome core is also attacked, at a slower rate^{30,31}. Staphylococcal nuclease cuts across both of the DNA strands³⁰, whereas other enzymes, such as pancreatic DNase, usually make single-strand cuts, so that the cutting pattern is somewhat obscured unless the digestion product is denatured³¹. Although the details of the process differ, all of the enzymes produce an array of fragments of discrete size. The pattern of single-strand cuts generated by pancreatic DNase is especially striking: when DNA from a partial digest of nuclei is denatured and subjected to gel electrophoresis, a series of discrete bands is observed, corresponding to DNA sizes that are integral multiples of ten nucleotides³¹ (Fig. 1, left). It is evident that there are discrete cleavage sites within the nucleosome; the way in which

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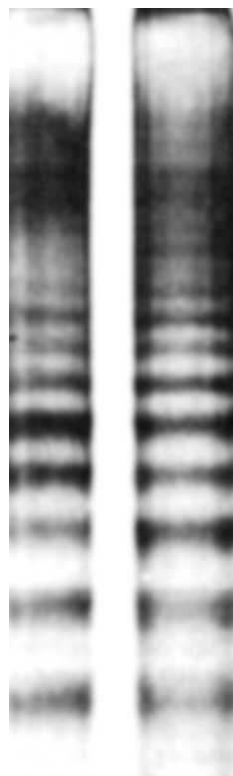


Fig. 1 The products of internal cleavage of nucleosomal DNA. Duck erythrocyte nuclei were digested with nuclease and the DNA purified, denatured, and electrophoresed on a polyacrylamide gel. Left channel: Pancreatic DNase (DNaseI) digest, 23% of the nuclear DNA acid soluble (after ref. 31). Right channel: Spleen acid DNase (DNaseII) digest, 60% acid soluble. Migration is from top to bottom. The dark-stained bands correspond to single strand DNA fractions separated by 10 nucleotide intervals in the approximate range 140–30 nucleotides. (From ref. 60.)

band intensities vary shows that the sites are not equally accessible.

The electrophoretic pattern is a map of the relative frequency of a given separation between sites, but it tells us nothing about their absolute location. For that, the nuclease digestion experiment is repeated starting with nucleosome core particles that have been labelled *in vitro* with ^{32}P at the 5' termini of the DNA chains. The electrophoretic pattern of radioactively labelled fragments reflects the distance of susceptible sites from a fixed point on each chain. This experiment has been carried out independently in three laboratories with roughly comparable results^{32–34}; there is general agreement that the sites 30, 80, and 110 nucleotides from each 5' terminus are quite insensitive to attack by pancreatic DNase. The absence of any fragments of these sizes means that each chain of the duplex is resistant to attack at these distances from its 5' terminus. This suggests a symmetrical distribution of protein–DNA contacts as a function of distance from the 5' termini of the antiparallel strands^{32,33}.

Such a symmetrical distribution of cleavage sites would be expected in a structure containing a dyad axis, as has been proposed on the basis of histone interaction studies³⁵. The possibility of this two-fold symmetry in the histone octamer itself has led to the proposal that the nucleosome not only contains a dyad axis, but is capable of unfolding into two symmetrical half-nucleosomes, perhaps as part of some mechanism of replication or transcription³⁶. A dissociation pathway of this kind is suggested by studies of histone interactions in the absence of DNA. When nucleosomes are dissolved in 2 M NaCl, the liberated core histones (H2A, H2B, H3, and H4) have been reported to form 'heterotypic tetramers' containing one molecule of each histone species³⁷. However, there is an unresolved disagreement about this observation,

since other workers report that the predominant species in 2 M NaCl is an octamer, rather than a tetramer³⁸.

Regardless of the nature of these histone interactions, the idea of nucleosome unfolding is supported by a number of studies of the effects of ionic strength and denaturing solvents on the nucleosome's physicochemical properties^{39–42}. Furthermore, it has recently been reported that at low ionic strength, the nucleosome as seen in the electron microscope appears to split into a pair of smaller particles⁴³. For example, the normal SV40 minichromosome, which bears 20–24 nucleosomes⁴⁴, is converted into a structure with a slightly greater contour length, but with 40 to 50 beads, each about 3/4 the diameter of the normal nucleosome.

Further evidence for a bipartite nucleosome structure comes from studies of the action of spleen acid DNase, a DNaseII, on chromatin. This enzyme can act on nuclear DNA to generate fragments that are multiples of 100 base pairs in length, suggesting that in addition to preferred cleavage sites between nucleosome cores, there may be another in the middle of each core. Although no corresponding nucleoprotein particle has been isolated, the results are consistent with the presence of a site of symmetry within the nucleosome. Preferential cleavage at this site is not seen in isolated nucleosomes, and only occurs in certain ionic conditions in nucleosome oligomers or purified chromatin; the inference can be made that nucleosome interaction and higher order structure are connected with this phenomenon.

Although the existence of a dyad axis in the nucleosome is not proven, it is certainly reasonable to introduce this symmetry element into any tentative model, and Finch *et al.*²⁹ have done so (Fig. 2). In addition, they have made use of the nuclease digestion studies mentioned earlier^{29,33}, which show that most sensitive sites, measured from a labelled 5' terminus, occur in pairs separated by 80 nucleotides along a given chain. Such a pattern could be explained if each superhelical turn contained exactly 80 base pairs, so that the backbone elements of adjacent turns were closest to one another when separated by 80 base pairs along the double helix. Since in this model the adjacent turns are separated by only 28 Å along the nucleosome surface, it is argued that the local environment, and hence nuclease sensitivity, would be the same for two elements separated by the length of a supercoil turn (Fig. 2).

This analysis is an attempt to explain the modulation of site accessibility, but avoids the more difficult question: what is the origin of the restriction that confines nuclease site separation to multiples of ten nucleotides? Two kinds of explanation have been proposed; both are related to the 10 base pair helical pitch of B-form DNA fibres, and both postulate that this periodicity exists in nucleosomal DNA. In the "kinky helix" models^{46,47}, linear segments of B-form DNA are discontinuously bent at intervals of ten base pairs; the nuclease-sensitive sites are at the bends. A second model for DNA folding involves continuous bending of the DNA in such a way that the double

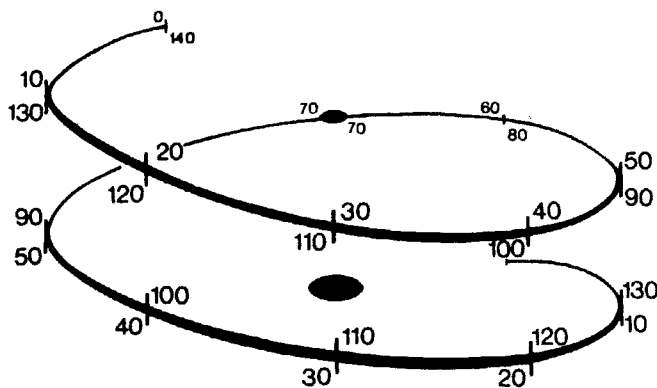


Fig. 2 Proposed superhelical path of DNA wound around the nucleosome core, showing nuclease cleavage sites and proposed dyad axis. (From ref. 29.)

helix makes one complete turn, relative to the nucleosome surface, for each ten base pairs. If a strand is accessible to nuclease only when it protrudes most from the nucleosome surface, nuclease-sensitive sites will occur once every ten nucleotides on each strand³¹. If cleavage occurred precisely at these sites, cuts on opposite strands would be separated by six or four nucleotides in a staggered arrangement. On the other hand, cuts occurring at kinks would be found either directly across from one another, or separated to give a stagger of ten nucleotides. These predictions can be tested directly by isolating double-stranded DNA from pancreatic DNase (DNaseI) digests. Wherever cuts on opposite strands are close together, fragments separate from each other, forming a series of small double-stranded molecules with single-stranded tails. The size of these tails, and thus the size of the stagger, can be determined by repairing the single-stranded region with a DNA polymerase, which extends all chains having recessed 3'-OH termini. It is found in this way that the cutting pattern corresponds to a stagger of 8 nucleotides (3'-OH recessed) and 2 nucleotides (5'-P recessed) between adjacent cutting sites on opposite strands^{48,49}.

This result is consistent with neither of the predictions described above. It can be made consistent with either by assuming that the site on the nucleosome recognized by the enzyme does not coincide with the cutting site, but is appropriately displaced in the 3' or 5' direction (Fig. 3). Further support for this idea comes from similar studies⁵⁰ of the mode of action of spleen acid DNase, a DNaseII. The nucleosome digest pattern of this enzyme is like that of DNaseI (Fig. 1), but detailed examination shows that in this case the cutting sites are staggered by six and four nucleotides (Fig. 3). Making use once again of labelling at the 5' terminus, the relative positions of the DNaseI and DNaseII cuts can be compared. It is found that the two sets of cutting sites are related to each other by a common dyad axis. Using somewhat different procedures, staphylococcal nuclease can be shown to cut at still another set of sites, which share the same dyad axis⁵⁰. It seems that the common recognition site is determined by the nucleosome structure, but the exact cutting point depends upon the enzyme.

Nucleosome folding and DNA supercoiling

Unfortunately none of this enables us to decide between kinked and continuously deformed models of DNA in the nucleosome. It is certainly possible to construct acceptable models of superhelical DNA by continuous deformation of the double helix^{29,61}. Estimates can be made of the energy required to bend DNA into a continuous supercoil with the dimensions of a nucleosome, using a well known relationship between the persistence length of such a stiff coil and its average bending force constant⁶². According to this model, about 20–28 kcal/mol of nucleosome are required to bend the DNA. The electrostatic forces which bind histones to DNA are very large at physiological ionic strength. The integrity of the folded nucleosome may therefore depend upon the attractive forces between histones within the nucleosome; if they are weak, the nucleosome will unfold. The free energy of interaction of histones in the nucleosome can be estimated, starting with free energy values measured in solution^{35,53}. It is found⁶² that one or two pairwise interactions of average strength would be sufficient to maintain the nucleosome in closed form. An unfolding pathway, such as that leading to half-nucleosomes, might involve disruption of just such a small number of interactions, and permit a delicate balance between folded and unfolded forms. It should be noted, however, that such an unfolding may not be an obligatory pathway for nucleosome assembly or disassembly: a particle with most of the physical properties of a nucleosome can be generated⁶⁴ by combining a DNA fragment 140 base pairs long with a histone octamer in which the proteins have been crosslinked, preventing histone dissociation and presumably interfering with unfolding as well.

Whether the DNA of the nucleosome is kinked or continuously deformed, every proposed structure must not only have

dimensions appropriate to the nucleosome, but must also satisfy the topological criteria implicit in experiments⁵⁵ which demonstrate the ability of the core histones to induce supercoiling. These experiments show that each octamer of core histones induces supercoiling in closed circular DNA equivalent to a change in linking number (ΔLk) of about -1 , reflecting additive contributions from changes in both the writhing number and the twist of the DNA^{56,57}. The simple models of kinking or continuous deformation described above fix the final state of twist of the DNA; similarly, the writhing number can be calculated for the proposed superhelical path of the double helix along the surface of the nucleosome²⁹. Thus, ΔLk can be calculated for the conversion of relaxed to nucleosomal DNA.

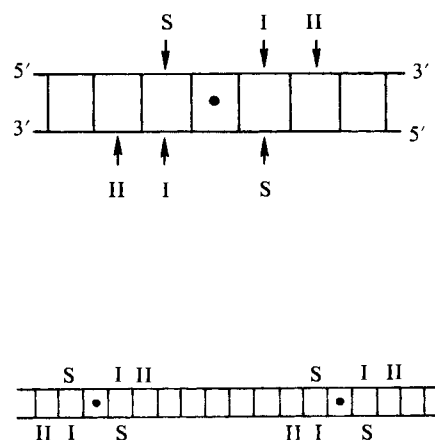


Fig. 3 Relative locations of the cleavage sites on nucleosomal DNA of pancreatic DNase (I), spleen acid DNase (II) and staphylococcal nuclease (S). The dot represents the local dyad axis of the cuts. (From ref. 50.)

If the fully relaxed conformation has ten base pairs per turn, as is generally assumed for B-form DNA, the agreement between the calculated and observed values of ΔLk is not good. It has been suggested²⁹, on the basis of recent theoretical calculations of DNA conformation, that B-form DNA in solution (though not in fibres) may in fact have about 10.7 base pairs per turn. This would certainly remove the discrepancy in ΔLk by changing the starting value of the twist, leaving the nucleosome model unchanged. At this point, none of the experimental observations concerning nucleosome structure, DNA supercoiling, or DNA conformation in solution is sufficiently precise or incontrovertible to permit a decision as to which is in error.

Role of arginine-rich histones

Nucleosomes can be reassembled by mixing DNA and histones under controlled conditions. The reconstituted particles have the characteristic beaded appearance in the electron microscope⁶⁵, and give typical subnucleosome fragment patterns when digested with nucleases^{30,58}. Recently, the four core histones have been reconstituted with DNA 140 base pairs in length, to give a homogeneous preparation of particles with sedimentation coefficient identical to that of native nucleosome cores⁵⁹.

The availability of reliable reconstitution methods makes it possible to examine the role of each histone component in the organisation of the nucleosome, by systematically omitting one or more from the reconstitution procedure. In this way it has been found that the arginine-rich histones, H3 and H4, are necessary and sufficient for the generation of a nucleosome-like complex. When H3 and H4 alone are reconstituted with DNA in appropriate conditions, the resulting complex can be digested with nucleases to give subnucleosomal DNA fragments identical in size (though not in relative abundance) to those produced when nucleosomes are digested^{58,60}. Such H3–H4 complexes also show a characteristic resistance of the histones to attack by trypsin or chymotrypsin, seen also in nucleosomes⁶⁰.

Furthermore, X-ray diffraction patterns of fibres made from reconstituted complexes of DNA with H3-H4 are almost identical to those obtained from intact chromatin fibres^{61,62}. It has also been shown that these two histones alone are capable of inducing an extent of supercoiling in closed circular DNA approximately equivalent in weight (roughly twice the normal complement) to that induced by all four histones^{52,63,64}. No combination of histones that omits either H3 or H4 gives any indication of ability to induce structure^{52,58,60}.

Complexes of the arginine-rich histone pair with closed circular DNA appear in the electron microscope as a beaded string^{63,64}, although the bead diameter is somewhat smaller than that of a normal nucleosome. If DNA 140 base pairs long is used, isolated beads are seen⁶³. The beads sediment in the ultracentrifuge in a manner consistent with their compact structure^{50,63}. Though there is some debate about whether a tetramer⁶³ or an octamer⁵⁰ of H3-H4 is associated with such a folded bead, it is clear that the arginine-rich histone pair plays a central part in nucleosome formation, as first suggested by Kornberg³. The arginine-rich histones have been the most strictly conserved in amino acid sequence during evolution. They are also the most tightly bound of all the histones. The results so far obtained leave open the question of the role of the slightly lysine-rich histones, H2A and H2B, in completing the normal nucleosome structure. That role may not become apparent until more is known about the function of nucleosomes in biological processes.

Higher orders of structure

The nucleosome must also be involved in the higher orders of structure that fold the DNA into the extremely compact form found in the nucleus. Attention has been concentrated on the first two levels of organisation, a 'thin' chromatin filament, 100 Å in diameter, and a thicker fibre, with a diameter of 200–300 Å. The thin fibre is almost certainly a linear array of nucleosome cores in contact with one another. The thick fibre seems to be generated by coiling of the thin fibre.

Neutron diffraction studies of the 300 Å chromatin fibre suggest that the nucleosomes are arranged in a solenoid with a 100 Å pitch, a diameter of 300 Å and a 100 Å hole down the central axis⁶⁵. The pitch is presumably determined by the side-by-side packing of nucleosomes on adjacent turns of the solenoid. Solenoids of these dimensions are also seen in electron micrographs of nucleosome oligomers⁶⁶, but only when magnesium ion is present. In its absence, only the 100 Å thin filament is visible. It is easy to build models^{66,67} in which strings of spherical beads are packed to form the solenoidal structures, although some modification will be necessary to take into account the correct, non-spherical nucleosome shape.

There is good reason to think that interactions between nucleosomes are modified or stabilised by the lysine-rich H1 histones. These histones are bound to chromatin at least in part through attachment to the DNA spacer region between nucleosome cores. During staphylococcal nuclease digestion, H1 histone is liberated from monomer nucleosomes as the DNA size is reduced from 200 base pairs to 140 base pairs^{68–70}. A DNA fragment about 35 base pairs long appears simultaneously, with H1 bound to it⁷¹.

The presence of H1 may have a direct effect upon chromatin structure. For example, SV40 minichromosomes containing H1 appear as compact structures in the electron microscope. Salt extraction, which should remove H1, allows the structure to open up into the more familiar beaded forms^{72–76}. Similarly, if well fractionated nucleosome oligomers are stripped of histone H1, there is a change in the dependence of the frictional coefficient on oligomer size⁷⁰, consistent with stabilisation of compact structures by H1. Studies of the dependence of H1 binding on the size of nucleosome oligomer show that there is a steady increase in affinity up to the octanucleosome, and no further size dependence beyond that⁷⁷. This suggests that octanucleosomes are capable of forming a stable unit of higher order structure: electron micrographs of oligonucleosome fractions

containing H1 reveal the presence of spherical structures about 200 Å in diameter, which have been termed 'superbeads', and seem to contain 6 to 10 nucleosomes each⁷⁷. The relationship of these structures to the superhelically wound oligonucleosome fibres seen earlier is uncertain. It has been pointed out⁷⁷ that exposure of chromatin to very low ionic strength solvents disrupts the higher order 300 Å chromatin fibre, and disturbs the size-selective binding of H1 noted above, perhaps by disruption of cooperative H1 interactions; variations in structure may arise from such rearrangements.

It is not clear exactly how H1 contributes to compaction of oligonucleosomes. The mode of binding of H1 to either negatively or positively wound superhelical DNA is different from its binding to the relaxed circular form⁷⁸. H1 may form bridges between superhelical turns, and indeed it has recently been shown that when H1 is added to H1-depleted SV40 minichromosomes, some H1 molecules link non-adjacent nucleosomes⁷⁵. H1 molecules may also connect spacer regions on either side of a single nucleosome⁷⁹. Either mode of binding could permit H1 molecules, if their termini were close to each other, to be polymerised by chemical cross-linking reagents, a phenomenon that has been observed⁸⁰.

Of all histones, H1 has the most variation in amino acid sequence among histone subspecies, as well as the largest number of variants. It is reasonable to speculate that the size of the variable spacer region between nucleosome cores is correlated with the subspecies of H1 bound to it; there may be a relationship between the size of the spacer and the number of basic residues on the H1 molecule^{10,14}. It has also been suggested^{81,82} that variation in the amino acid composition of histones H2A and H2B^{83–85} could alter contacts between nucleosomes, and affect the size of the spacer. Whatever the determinant of the spacer length, the thin filament and higher order structures must be capable of accommodating variable lengths of DNA in the region between nucleosomes. It can be inferred from nuclease digestion studies that in at least some cases the lengths of these regions are integral multiples of ten base pairs¹⁷, and may also have regularly spaced accessible sites within them when H1 is present. Worcel⁸³ has proposed a model of the thin chromatin filament, 100 Å in diameter, in which adjacent nucleosome cores are in contact, but the cores can be rotated with respect to one another to permit inclusion of a variable length of spacer DNA without altering the diameter of the fibre. The spacer DNA is not in close contact with core proteins, but is assumed to be complexed with H1, in a way which stabilises supercoiling of the thin filament to form a 200–300 Å fibre. Furthermore, still higher order coiled structures can be formed from this fibre in such a way that the spacer DNA is always at the outside, and accessible to nucleases.

Such models, though they may turn out to be wrong in detail, provide important tools for thinking about higher orders of structure which nucleosome packing must generate, and which we have only begun to examine experimentally.

Chromatin structure and biological activity

The information we have so far obtained about chromatin structure clearly relates to DNA packaging in the eukaryotic nucleus. There is no reason to doubt the tenet of molecular biologists that knowledge of structure leads to understanding of function; what we now know about chromatin structure is simply not enough to permit the connection to be made. Perhaps for that reason, many of the questions now being asked about the role of histones in the biological activity of chromatin are little different in form from those asked four years ago⁸⁶, except that they are cast in terms of nucleosomes rather than in terms of more poorly defined histone complexes.

The obvious first task is to find out what happens to nucleosomes during DNA replication and transcription. In the case of replication, new histones are synthesised as well as new DNA, and new nucleosomes are made. Are the new histones segregated or mixed with the old? In a recent set of experiments⁸⁷, the fate of newly synthesised histones in chick myoblasts was traced by

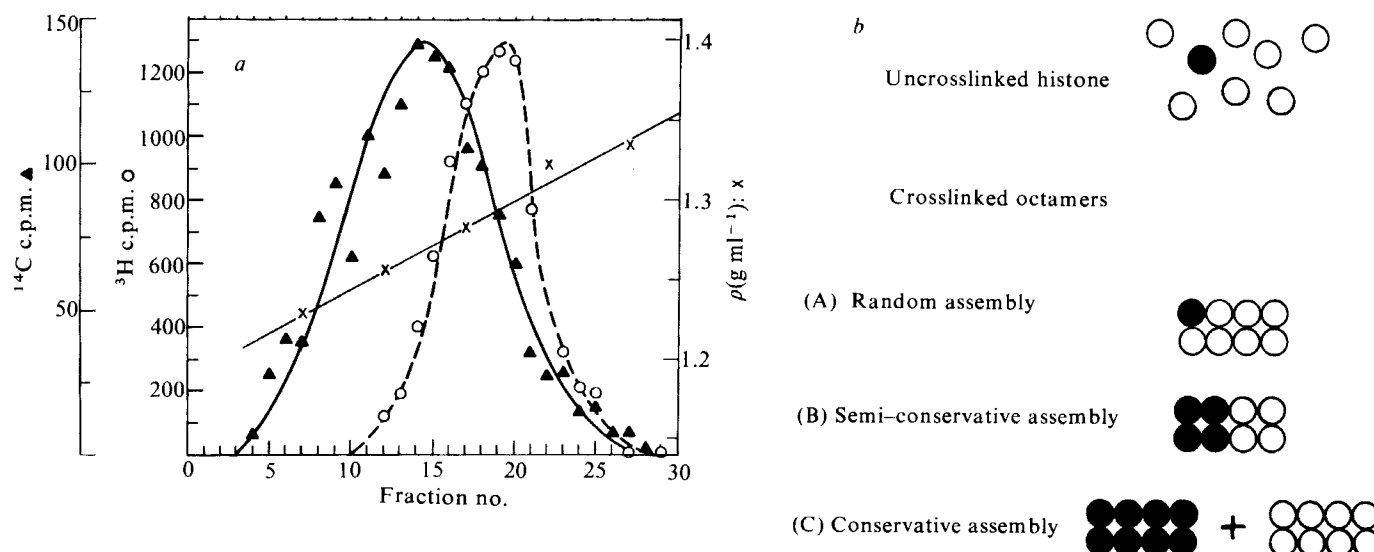


Fig. 4 *a*, Buoyant density centrifugation of newly synthesised histones crosslinked to form octamers. Cells were labelled for 1 h in dense amino acids and ^3H -lysine. Chromatin was prepared by mild nuclease treatment of nuclei. After crosslinking the histones with Lomant's reagent (dithiobis (succinimidyl propionate)), the sample was banded to equilibrium on a caesium formate-guanidinium chloride gradient, together with uncrosslinked, ^{14}C -lysine labelled, light histone marker. The density of the ^3H -labelled peak corresponds to octamers containing only dense amino acids. (From ref. 87.) *b*, Possible modes of assembly of newly synthesised histone octamers. The data in *a* support model C. (From ref. 87.)

pulse labelling them with ^3H -lysine and dense (^{13}C and ^{15}N labelled) amino acids. The nucleosome monomers were isolated from the cells, and the histones within each nucleosome cross-linked chemically. The crosslinked histones were freed from DNA and their density determined by isopycnic centrifugation. Although newly synthesised histone represented only one-seventh of the total histone, all the newly synthesised molecules were found to band in the fully dense position, showing that crosslinking within the octamer had not joined old and new histones to each other (Fig. 4). This demonstrates that newly synthesised histones form completely new nucleosomes, rather than forming mixed octamers with old histone.

By using modifications of this experimental approach, it is possible to show⁸⁷ that newly synthesised octamers tend to be located near other new octamers, confirming the model of non-random distribution of new nucleosomes that had been deduced earlier by less direct experimental methods^{88,89}. It can also be shown that individual histone octamers preserve their integrity (that is, segregate conservatively) through at least three or four rounds of replication⁸⁷. Other evidence suggests that such a conservative segregation mechanism governs the behaviour of long sequences of adjacent nucleosomes⁸⁷.

These experiments do not examine the way in which newly made histone interacts with newly made DNA, and in fact there are conflicting reports in the literature (see ref. 87) as to whether new histone distributes randomly, or segregates in association with new DNA. If the histone distribution is in fact non-random, the way is open to a variety of mechanisms which could, in principle, preserve the identity and sequence of nucleosomes in the chromatin of a stem cell line, while allowing diversity in daughter DNA duplexes.

Since histone octamers stay together *in vivo* through several generations, there is no need to invoke mechanisms in which the nucleosome disassembles as part of the replication process, though such mechanisms are not excluded provided that old subunits are able to find each other again. It remains to be determined how newly made nucleosomes choose the particular daughter helix to which they will bind. Whatever the role of nucleosomes in replication, it seems unlikely that their presence would result in a process simpler than that which occurs in prokaryotes⁹⁰. In studying replication in eukaryotes, it would be

reasonable to expect a complicated series of enzymatic reactions, to which nucleosomes have adapted.

Perhaps because the analogous reaction in prokaryotes is better understood, studies of chromatin transcription far outnumber studies of replication. It has proved difficult to construct a satisfactory transcription system *in vitro* that sheds light on the mechanism *in vivo*, partly because the mode of action of eukaryotic RNA polymerases is not well known. The prominent exception is the system for transcription of the 5S RNA genes of *Xenopus in vitro*. Roeder and his colleagues⁹¹ have shown that selective asymmetrical transcription of the 5S gene can be achieved, provided that the proper polymerase is used (that is the class III eukaryotic RNA polymerase, responsible for 5S gene transcription *in vivo*), and provided that the template is chromatin rather than protein-free DNA. Recent results⁹² suggest that reconstituted chromatin may be effective as a template, furnishing a tool for studying the role of the various components of chromatin in regulating transcription of these genes.

Most investigations of chromatin transcription *in vitro* have used bacterial RNA polymerase, and should be regarded as studies not of transcription, but of chromatin structure, using the polymerase as a probe. Although there have been numerous reports of selective gene-specific transcription of chromatin by bacterial polymerase, the approach has not so far yielded a great deal of information about chromatin structure, probably because the methods used are tedious and subject to numerous potential artefacts⁹³⁻⁹⁵. It has recently been found, for example, that when endogenous mRNA is present as a contaminant in chromatin preparations, it can be used as a template by *E. coli* RNA polymerase to make an RNA strand complementary to the message, which forms a duplex with it^{94,95}. Certain techniques for distinguishing and isolating newly made RNA, such as incorporation of mercury-substituted nucleoside triphosphates, will lead to isolation of the duplex, and hence accidental purification of the endogenous message^{94,95}. The endogenous message sequences will subsequently be detected in the assay and ascribed erroneously to synthesis *de novo*. Complications such as these have made it difficult to use bacterial RNA polymerases as probes of chromatin structure.

How can we obtain more information about chromatin

structure in the neighbourhood of a transcriptionally active gene? One useful approach is to ask whether the structure is like that of the bulk of chromatin with respect to its sensitivity to nuclease. The answer that is obtained depends upon the nuclease that is used.

When staphylococcal nuclease is used to digest transcriptionally active, non-ribosomal genes, the pattern of products is very similar to that generated by this enzyme when it attacks the bulk of chromatin. For example, globin genes in avian reticulocytes are protected against exhaustive digestion by staphylococcal nuclease to the same extent as all other chromatin DNA^{50,96,97}. This parallel behaviour is also observed earlier in digestion: Lacy and Axel⁹⁸ have isolated nucleosome monomers, sedimenting at 11S, from partial staphylococcal nuclease digests of avian reticulocyte nuclei, and find that the abundance of globin gene sequences in the DNA of this fraction is identical to the genomic abundance. Thus the globin genes in reticulocytes, which are active in producing globin mRNA, are packaged in structures possessing the same repeat as the nucleosome. Similar results have been obtained⁹⁹ for the abundance of ovalbumin sequences in purified nucleoprotein monomers from hen oviduct nuclei.

The distribution of ovalbumin sequences has also been examined by fractionating the partly digested nuclear DNA rather than the nucleoprotein oligomers¹⁰⁰. Early in digestion, most of the ovalbumin genes are present in DNA fragments that are multiples of a subunit about 200 base pairs long. But, the monomer DNA fraction is preferentially enriched in ovalbumin sequences. The apparent inconsistency with the earlier observations⁹⁹ that 11S nucleoprotein monomers are not similarly enriched would be resolved if some ovalbumin sequences 200 base pairs long were not derived from 11S particles. Proof of this hypothesis will require a careful accounting for the fate of all ovalbumin genes, both as monomer DNA and nucleoprotein, in the course of the digestion.

susceptibility of chromatin from adenovirus-transformed hamster cells, the easily digestible adenovirus sequences seem to correspond to that portion of the virus from which mRNA is transcribed; the other viral sequences are resistant.

These results reveal a correlation between DNaseI sensitivity and a potential for or history of gene activity. Response to the nuclease does not necessarily depend upon how often the gene is being transcribed. For example, the globin gene retains its sensitivity in blood cells from 18-d-old chick embryos⁹⁷, in which globin message synthesis has largely ceased. Similarly, gene sequences that are infrequently represented in the mRNA population are nonetheless DNase sensitive⁹⁹. If it is assumed that the abundance of an mRNA sequence reflects the number of RNA polymerase molecules bound to the corresponding DNA, it can be concluded that it is not the transcription complex that confers special sensitivity to pancreatic DNase. We do not know whether the structural differences detected by this enzyme are a cause or an effect of transcription.

What kinds of structure could confer such sensitivity? Protein-free DNA might be digested preferentially. However, the same sequences that are digested by DNaseI are resistant to staphylococcal nuclease in a way which indicates that proteins are present. It should be emphasised that the absence of preferential digestion by staphylococcal nuclease is not necessarily diagnostic of the presence of protein. For example, at very low ionic strength, the kinetic constants describing the action of this enzyme on chromatin and on naked DNA⁶⁰ are such that, in substrate excess, mixtures of chromatin and DNA will be digested at approximately equal rates. Thus, experiments which merely test the initial rate of digestion of a sequence may not, in certain conditions, distinguish between chromatin and DNA. For these reasons, the crucial observations with staphylococcal nuclease are the appearance of most of the transcriptionally active sequences in nucleosome-like repeats, and the appearance of the genomic abundance of these sequences in

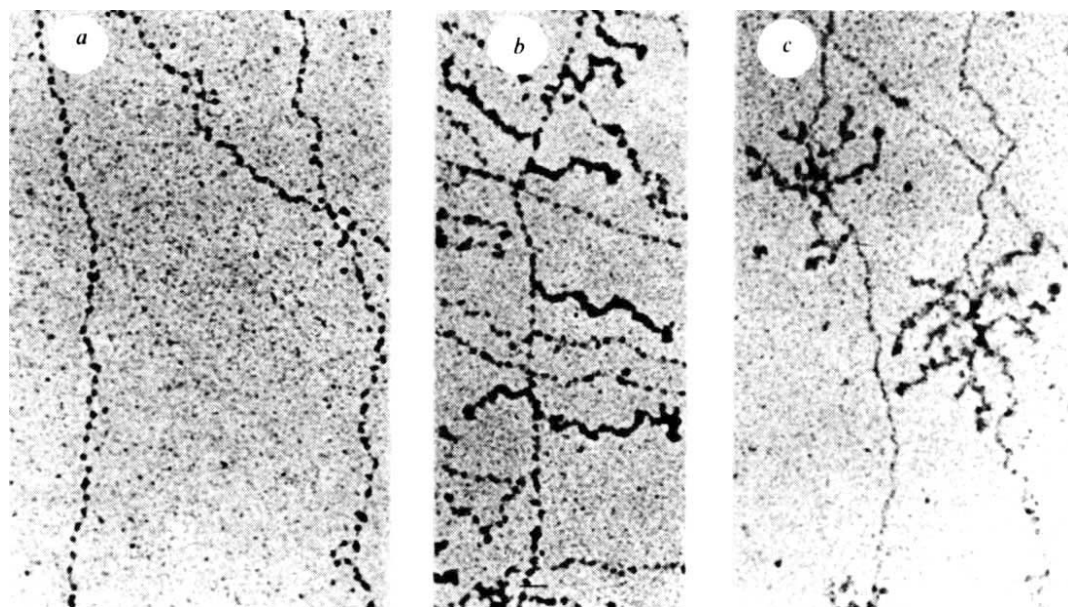


Fig. 5 Electron micrographs of chromatin from *Oncopeltus fasciatus*, enlarged about 67000 $\times$. a, Beaded chromatin from regions devoid of ribonucleoprotein (RNP) fibres. b, Beaded chromatin between nascent non-ribosomal RNP. c, Unbeaded chromatin of newly induced ribosomal transcription units. (From ref. 106.)

In contrast to the results with staphylococcal nuclease, the response of transcriptionally active sequences to digestion by pancreatic DNase (DNaseI) is strikingly different from that of normal chromatin. As first reported by Weintraub and Groudine⁹⁷, and subsequently confirmed in a number of laboratories, DNA sequences corresponding to active genes are digested preferentially. The list of susceptible sequences includes the globin gene in chick erythrocytes⁹⁷, the ovalbumin gene in hen oviduct¹⁰¹, and endogenous avian tumour virus sequences in chicken DNA⁹⁷. In a similar study¹⁰² of the pancreatic DNase

'limit digests'^{50,96}, which contain only fragments of subnucleosome size. The former observation shows that the DNaseI sensitive regions are packaged with proteins in a way that at least mimics nucleosomes; the latter observation strongly suggests that the proteins in question are indeed the nucleosomal complement of histones. To reconcile this conclusion with the observed sensitivity to pancreatic DNase, it must be assumed⁹⁷ that the nucleosome structure is modified or opened in a way that permits pancreatic DNase, but not staphylococcal nuclease, to detect the difference.

It should be noted that although the pattern of sensitivity described above has been observed for most of the active genes so far examined, there are exceptions. At least one integrated viral sequence has been found to be sensitive to both pancreatic DNase and staphylococcal nuclease¹⁰⁹. There is also some evidence both from nuclease digestion¹⁰⁴ studies and electron microscopy that actively transcribed ribosomal genes may not be like the other active genes discussed above. Ribosomal genes that are undergoing transcription are identified easily in the electron microscope by the characteristic tree-like structures formed by the growing ribonucleoprotein fibres, which extend from the DNA¹⁰⁵. Unlike the bulk of chromatin, no 'beads' are visible on these genes. Furthermore, since the length of DNA in each ribosomal gene is known, the DNA packing ratio can be measured, and it is found to be 1.2 (refs 106 and 107). Thus, the DNA of the transcribed ribosomal genes is only slightly less extended than normal protein free-DNA. This does not mean that proteins are necessarily absent from the ribosomal genes, since the mean diameter of the ribosomal chromatin fibre is about 73 Å.

Electron microscopists have also examined non-ribosomal genes undergoing transcription. Although subunit structure is not always visible¹⁰⁸, there are at least some cases in which a beaded structure has been reported¹⁰⁶. The difficult step in such observations is the identification of the active region. A tree-like structure of growing ribonucleoprotein chains can be found, and although it is less regular than the structure found on ribosomal genes, a convincing argument can be made that the chains arise from transcription of a delimited segment of DNA¹⁰⁶. Examination of these non-ribosomal 'transcription units' reveals the presence of beaded structures on the chromatin between ribonucleoprotein chains (Fig. 5). The diameter of these beads, about 13.5 nm, is similar to that found for nucleosomes under similar preparative conditions. The number of beads per unit length of chromatin within the transcription unit is about 70% that found in the bulk of chromatin¹⁰⁶. Recent experiments have shown that these regions react with antibodies to histones H2B and H3¹⁰⁹.

The electron microscope studies support many of the conclusions derived from the nuclease digestion experiments, but have not so far revealed the structural basis of sensitivity to pancreatic DNase. The sensitivity could arise from an alteration within each core particle (for example, to permit formation of half-nucleosomes), perhaps as a result of chemical modification of the histones, or it could reflect a perturbation of the higher-order packing of the nucleosomes, perhaps connected with loss or modification of histone H1 molecules.

The view we presently have of chromatin structure is still largely static. We do not yet understand any of the many conformational changes that must take place during the cell cycle both in individual nucleosomes, and in the higher order structures they form. Some of these changes may arise from modifications in composition. We know that the histones are chemically modified at various times during the cell cycle. Furthermore, during development, subspecies of certain histones are synthesised that differ slightly in amino acid sequence^{83,84} and might function differently in the nucleosome. Chromatin structure must also involve components that have been excluded from this discussion: the proteins that are not histones. The so-called high-mobility group (HMG) proteins¹¹⁰, for example, are found in abundance associated with chromatin¹¹¹. Another protein, ubiquitin, is found covalently coupled by an isopeptide linkage to histone H2A, in some cases modifying one in five H2A molecules^{112,113}. There are many other proteins, among them the polymerases themselves, that react with and may modify chromatin structure, and we may suppose that there are smaller molecules as well that have that capability. As we begin to understand the chemistry of these many reactions, the relationship between structure and mechanism may emerge.

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articles

Volume and extent of the Minoan tephra from Santorini Volcano: new evidence from deep-sea sediment cores

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Analyses of tephra in abyssal piston cores collected during cruises of R/V Trident show that the Minoan eruption produced at least 28 km³ of tephra (13 km³ dense rock equivalent). A layer up to 5 cm thick must have been deposited on eastern Crete.

THE great Bronze Age eruption of Santorini (Thera) in the Hellenic arc is one of the largest known explosive eruptions in post-glacial time. The eruption, which occurred approximately 1500 BC, led to widespread tephra-fall in the Eastern Mediterranean and resulted in the formation of a caldera with an area covering 83 km² and a depth of 600–800 m. Apart from its volcanological significance, this Bronze Age eruption acquired added archaeological importance after Marinatos¹ advanced the theory that the wide-spread Late Minoan destruction on Crete was a consequence of the paroxysmal volcanic events on Santorini. The discovery of Minoan volcanic tephra in a few deep-sea sediment cores from the Eastern Mediterranean² demonstrated the large magnitude of the eruption and the extent of the tephra-fall, and excited closer examination of Marinatos' theory¹. In September 1975, 32 piston cores were collected from the Aegean Sea and parts of the Eastern Mediterranean (Fig. 1) during Cruise 172 of the R/V Trident. These coring traverses were designed specifically to study the distribution and dispersal of tephra downwind from Santorini volcano. We report here results of our study on the Minoan ash layer which provide new evidence on the magnitude and effects of the eruption.

Correlation and distribution of the Minoan tephra

The locations of the new cores, together with cores containing Minoan tephra from RV Trident Cruise 171 and Vema Cruise 10 are shown in Fig. 1a. The thicknesses of megascopically visible Minoan tephra layers are added together with isopach contours. Visible Minoan tephra was observed in 18 of the new cores. We have correlated the tephra layers not only from core to core, but also with the Minoan tephra sequence on Santorini itself by electron microprobe analyses of glass shards and pumice clasts. Table 1 shows mean analyses of the Minoan tephra as sampled on Santorini and mean analyses of glass shards from the Minoan layer in abyssal cores collected during the cruises of RV Trident and RV Vema. Figure 2a shows plots of FeO*–K₂O–CaO + MgO of microprobe analyses of glass in six of the largest pyroclastic deposits exposed in the caldera wall of Santorini. Figure 2b shows the same plots for five prominent megascopically visible tephra layers in the Trident and Vema core V10–58. We have also analysed many other smaller, less widespread layers on Santorini and in the cores and the Minoan tephra is always distinguishable on major element glass chemistry. The data in Table 1 and the figure demonstrate clearly that the major element glass chemistry of the Minoan tephra is sufficiently distinctive to facilitate easy identification of the ash. The data provide unambiguous intercore correlations and confirm earlier correlations^{2,3}. Doubts about the correlation of the Minoan tephra in deep-sea cores can now be discounted^{4,5}.

Our results on measured thicknesses of the Minoan tephra layer in the deep-sea cores are presented in an isopach map in Fig. 1a. The isopach contours are based principally on the observed thicknesses in the cores. We stress that the contours in Fig. 1a

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depict minimum present-day thickness of compacted megascopic tephra layers in the cores. The new data indicate an easterly to southeasterly dispersal of the tephra with the fall-out axis passing through Karpathos. An even more easterly trend of the axis cannot be ruled out, however, on the basis of the present evidence. Isopach studies of the initial plinian deposit on Santorini itself⁶ have previously indicated a southeast to east-southeast dispersal during the early stages of the eruption. The direction of the

dispersal axis has been further confirmed by our continuing grain size studies of the tephra: the coarsest grain size in a tephra layer at a given distance from the source occurs close to our inferred axis (such as core 172-9) and the size decreases southwards. The extent of the tephra dispersal to the north is unknown due to lack of cores, but our data clearly show that some tephra-fall occurred on Crete and that the recent suggestion that the fall on Crete was negligible⁵ is untenable.

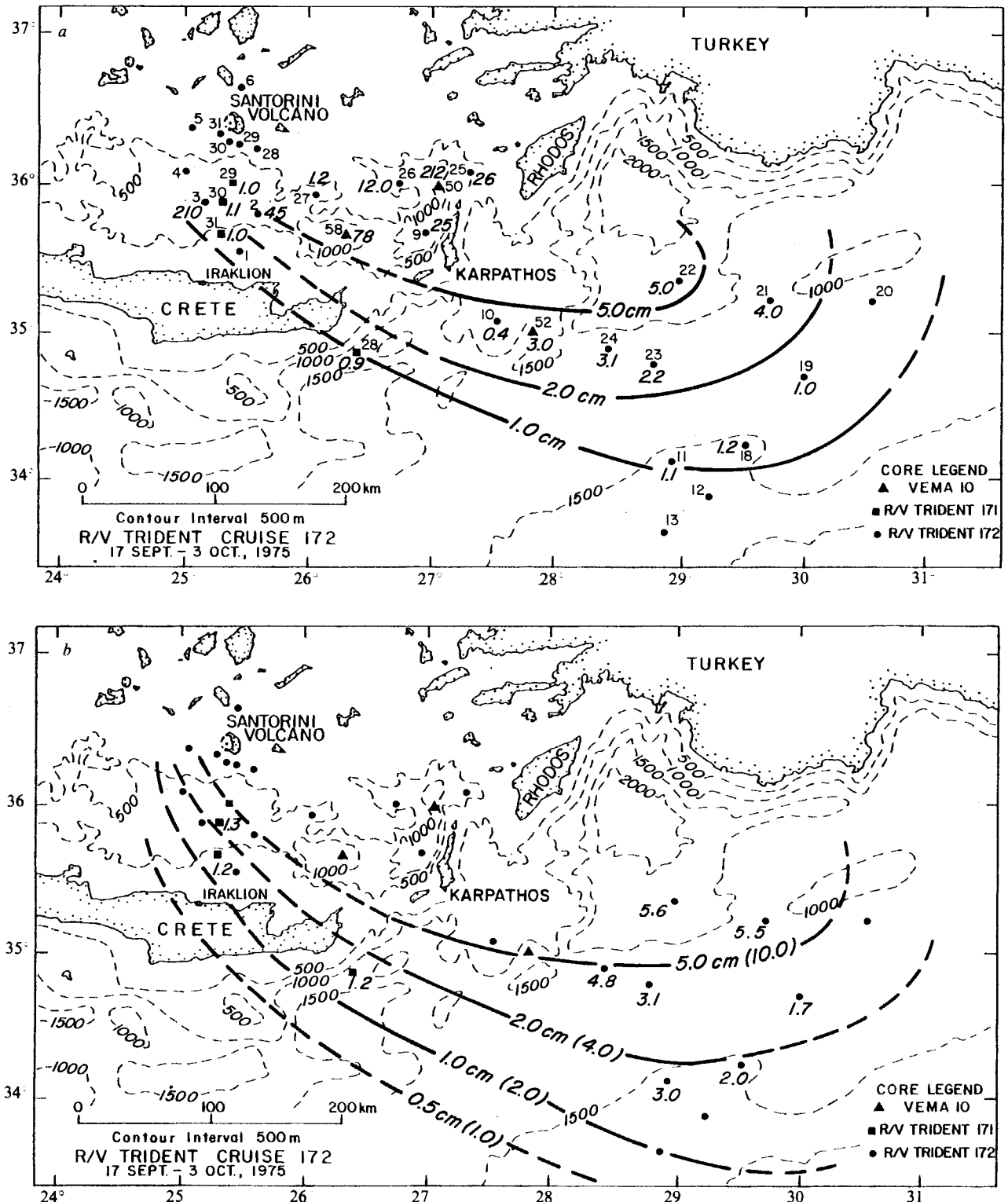


Fig. 1 Isopach maps of the Minoan tephra in Eastern Mediterranean cores. Isopachs are based on (a) the observed thickness (in cm) in the cores and (b) the adjusted thickness (in cm) to include the effects of tephra dispersal due to bioturbation. Note that in (a) the core numbers are given by small letters and the observed thicknesses in large letters. In b the numbers in parentheses on the isopach contours represent the thicknesses of freshly fallen tephra (before compaction) on land which are approximately twice those observed in the cores.

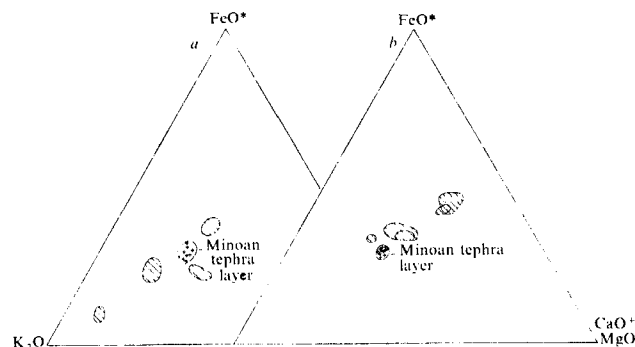


Fig. 2 FeO* (total iron) — K₂O — CaO + MgO plots of analyses of glass in tephra deposits from the Hellenic Island Arc. *a*, Six of the prominent pyroclastic layers in the caldera wall of Santorini are shown including the Minoan tephra layer. Each field is based on at least 10 analyses. *b*, Five widespread tephra layers found in cores from the Eastern Mediterranean are shown. The Minoan layer can be readily distinguished from the older Y-4 layer⁹ although the refractive indices are indistinguishable. Each field is defined by at least 12 analyses. The Minoan field in *a* coincides with that in *b*.

The RV Albatross cores collected by the Swedish Deep-sea Expedition⁷ are excluded from our data as we have been unable to obtain samples to correlate the alleged Minoan tephra layers in these cores. There is clearly some doubt over the correlations in the RV Albatross cores⁴: for example, core 192 south of Crete has a tephra layer in the upper part of the core with a thickness of 4 cm (refs 2,7) and has influenced previous reconstructions of tephra distribution and estimates of tephra thickness on Crete. This core lacks the distinctive 7,000 yr BP sapropel characteristic of many cores in the Eastern Mediterranean⁸. The stratigraphy in this core is therefore quite atypical of the strata associated with the authenticated Minoan tephra layer, but similar to the stratigraphy of tephra layers older than the 7,000 yr BP sapropel. An extensive tephra layer, known as Y-4 (after the recent stratigraphic nomenclature of Keller and others⁹), occurs beneath the sapropel in several cores and has a similar refractive index to the Minoan glass ($1,508 \pm 0.003$). This layer, however is different chemically (Fig. 2*b*). The upper layer in the RV Albatross cores could equally well be the Y-4 layer if the upper parts of the cores are missing. Because of these uncertainties we exclude these cores until unambiguous correlations for tephra in the Albatross cores are produced.

Controls on tephra thickness

Tephra layer thicknesses have to be interpreted with caution as both volcanic and marine processes may control the ultimate thickness observed in a core. At least three important marine processes modify tephra thicknesses: sediment-ponding, bioturbation and compaction. When a large volume of loose tephra accumulates during an eruption, slumping may occur to generate turbidity currents, grain flows and debris flows. The consequence of such sediment-ponding processes¹⁰ is an increase in layer thicknesses in basins and to thin or erase layers on submarine topographic highs and steep slopes. Bioturbation of tephra layers mixes the tephra with other sediments and can reduce visible tephra layer thicknesses, dispersing tephra in the overlying sediment. Finally post-depositional compaction can substantially reduce the thickness of the tephra layer compared to the freshly-fallen thickness.

Within the Aegean Sea the great thicknesses within the basins are due to repeated slumping during the eruption to produce successive turbidite units. Evidence for turbidite origin includes erosional bases and partial Bouma sequences, including graded tephra beds. Figure 3 shows the lithology of several units within the Minoan tephra layer in core 172-9 as well as profiles of carbonate and clay content, median grain diameter, and pumice, lithic and crystal contents. The two layers labelled D are both interpreted as turbidites as they contain abundant carbonate, clay and some rounded terrigenous quartz grains, mixed in with the tephra. By contrast the tephra layer in core 172-27 (Fig. 1*a*), for example, is only 1.2 cm in thickness despite the fact that the core is

only 80 km away and lies close to our suggested dispersal axis. This core is located on a steep slope and we attribute thinning of the layer in TR 172-27 to the slumping or erosion of much of the tephra. For these reasons none of the measured thicknesses of the Minoan tephra within the Aegean Sea have been used in our construction of isopach contours.

Bioturbation affects most tephra layers, but is of particular importance outside the Aegean Sea where the tephra layer becomes relatively thin. Using our technique¹¹ we have quantitatively analysed the dispersed tephra in continuous samples taken above and below the visible tephra layer in 11 selected cores. The volume of dispersed tephra has been determined and its equivalent thickness added to the thicknesses of visible ash in each core. Up to 65% of original tephra has been mixed upward by bioturbation. In the Indian Ocean a 15 cm ash layer from Toba west of Sumatra has lost some 5 cm of ash by bioturbation into overlying sediment¹² emphasising the importance of this process. Fig. 1*b* shows the corrected tephra thicknesses and revised isopach contours. Comparison of Fig. 1*a* and 1*b* illustrates that tephra layer thicknesses can be markedly altered by bioturbation. While the reconstruction does not alter the basic dispersal pattern of the Minoan tephra, the corrections clearly make a substantial difference to volume estimates.

The thickness of a compacted tephra layer in a deep-sea core is considerably less than the thickness of freshly fallen tephra on land. We have studied the effect of post-depositional compaction on tephra layer thickness by experimental resedimentation of dry Minoan tephra from the cores. The results of three experiments in air show that the compacted tephra layers are equivalent to only approximately 50% of the thickness of resedimented dry tephra of equivalent weight and grain size. These results are in close agreement with our findings on the 1902 tephra of the Soufriere of St Vincent¹³. This eruption resulted in 10 mm of tephra fall on Barbados, whereas gravity cores recovered a 5-mm tephra layer in the adjacent Tobago Trough. Thorarinsson¹⁴ has reported similar relations from historic tephra layers in Iceland. In Fig. 1*b* we also show our estimates of the original thicknesses of freshly fallen tephra and reconstructed isopach contours. This procedure reveals the likely thicknesses of tephra on Crete and adjacent islands, although other factors causing tephra compaction under the deep-sea environments were not taken into account.

Eruption sequence and tephra volume estimates

Volcanological considerations prevent simplistic interpretations of the data. Recent studies of the Minoan deposits on Santorini^{6,15} indicate three main phases in the eruption: (1) the plinian phase producing a coarse tephra fall deposit; (2) a phase of phreatomagmatic activity producing interstratified fine grained and poorly-sorted tephra fall deposits and base surge horizons overlain by mud-flow deposits; and (3) a pyroclastic flow phase producing many non-welded ignimbrite flow units, intra-formational flood deposits and very fine grained co-ignimbrite

Table 1 Microprobe analyses of glass in Minoan tephra from Santorini and the deep-sea cores

Oxide	Tephra on land	σ	Tephra in cores	σ
SiO ₂	70.60	(0.18)	71.40	(0.10)
Al ₂ O ₃	13.60	(0.06)	13.50	(0.14)
FeO*	1.74	(0.06)	1.74	(0.05)
MgO	0.24	(0.02)	0.25	(0.02)
CaO	1.35	(0.01)	1.32	(0.05)
K ₂ O	3.12	(0.04)	3.19	(0.06)
Na ₂ O	5.07		4.98	
TiO ₂	0.26	(0.02)	0.27	(0.01)
Total	95.98		96.65	

The table shows the mean of six analyses for the land-based sample, and the mean of three analyses for the samples from cores, together with the standard deviation (σ) for each element oxide. All are expressed in weight %. Total iron is expressed as FeO*. All analyses were performed on a JEOL JXA-50A electron microprobe, with accelerating voltage of 15 kV, beam current of 0.0125 μ A, specimen current of 0.0100 μ A, and a beam diameter of 5–10 μ m. The rate of sodium loss during analysis was measured as 50% of original concentration in 30 s. The quoted values for sodium have been corrected accordingly.

tephra fall deposits¹⁶. Each of these phases produced a vertical convective eruption column and air-fall ejecta from each phase probably has its own dispersal pattern and grain size characteristics. Complexities in tephra dispersal have been observed in other eruptions such as Karmai (1912)¹⁷ and the Mount Mazama eruption, Crater Lake, Oregon¹⁸, which formed products comparable to the Minoan Eruption.

The tephra layer in core 172-9 (Fig. 3) compares with the stratigraphy observed on Santorini. A coarse, ungraded, well-sorted crystal-rich layer, 3.5 cm thick (B in Fig. 3) occurs at the base with a sharp upper boundary and is overlain by several fine grained layers (C-F). Two of the normally graded layers with erosional basal boundaries (Interval D) are turbidites. Evidence of mixing with local sediment is absent in layer B and it is interpreted as a primary air-fall layer buried by the turbidites and later fine-grained tephra fall. Although 165 km from source layer B lacks the fine grain sizes which are abundantly present in the tephra fall layers produced by phases (2) and (3) on Santorini itself⁶. Thus layer B is interpreted as correlating directly with the plinian layer (phase 1) on Santorini whereas the upper layers are predominantly composed of phase (2) and (3) ejecta probably mixed with minor amounts of phase (1) material during slumping.

Figure 4 shows the relation between thickness and distance in several well-documented eruptions together with the estimated volume of tephra in each case^{14,18-21}. If layer B (Fig. 3) represents the primary thickness of the plinian layer an approximate curve can be drawn for the Santorini plinian deposit, using the near source data⁶ and core TR 172-9. The plinian deposit falls between the Toluca deposit¹⁹ in Mexico and the Mount Mazama plinian deposit, Oregon¹⁸, suggesting an approximate volume of

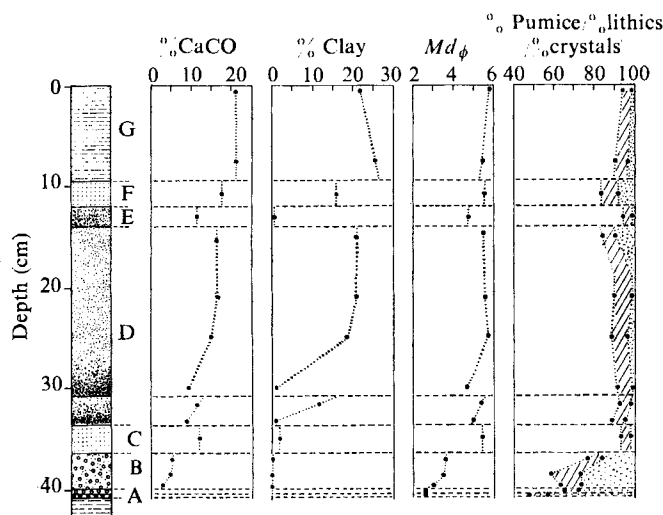


Fig. 3 Lithology of the Minoan tephra layer in core TR 172-9 showing sediment-ponding processes. From bottom to top: a, grain-flow deposit; b, plinian air-fall layer; c, fine tephra-fall unit; d, two repeated ash turbidites; e, and f, laminated tephra layers winnowed by bottom currents and g, pelagic muds admixed with tephra. Percent carbonate and clay, median grain size (Md_ϕ) and the weight percentage of pumice and glass shards (clear), lithics (hatched) and crystals (stippled) in grain size fractions greater than 63 μ m.

5–8 km³ for the ejecta in this phase. To the east of Karpathos the tephra layer in all cores consists of a single layer and evidence of a multiphase eruption is absent. One possibility is that the plinian layer has thinned to such an extent that it is undetectable or even absent. The tephra layers at 350–500 km distance would then represent the equivalent of phases (ii) and (iii) of the eruption.

Whether or not the above interpretations are correct, the thicknesses east of Karpathos can be used extrapolated back to Santorini to give an approximate guide to the overall magnitude of the eruption, and to indicate the possible location of isopach contours in the Aegean (Fig. 4). The maximum thickness of all the air-fall layers on Santorini is about 15 m. This estimate is for the air-fall components of all three phases: individually the plinian pumice layer has a maximum thickness of 5.5 m, the fine-grained

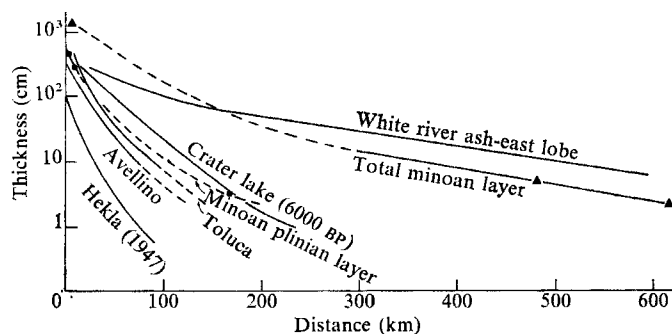


Fig. 4 Relationship between thickness and distance from source along the dispersal axis in several tephra fall layers, for comparison with the Minoan layer. Volumes of ejecta reported by the authors on each layer are Hekla¹⁴: 0.17 km³; Aveellino pumice deposit, Vesuvius²⁰: 2.1 km³; Toluca deposit, Mexico¹⁹: 3.5 km³; Crater Lake airfall deposit, Oregon¹⁸: 15 km³; White River Ash²¹: 25 km³; Minoan tephra: 28 km³. Curves are shown for the Minoan plinian deposit (●), based on the interpretation of TR172-9 and for the compacted thickness of the total Minoan tephra layer (▲) using the adjusted isopach contours in Fig. 1b.

air-fall and base surge ashes a maximum thickness of 6.5 m and the co-ignimbrite air-fall ash deposits a maximum thickness of 3 m. We have used the data in Fig. 1b and the approximate distance/thickness curve in Fig. 4 to estimate the volume of tephra. We have assumed that the dispersal axis passes through Karpathos and that the volume of ejecta south of the axis is matched by an equivalent volume north of the axis. The volume of tephra on Santorini itself, assuming an average thickness of all tephra of 15 m over 200 km², is 3 km³ and we estimate the total minimum volume of the compacted deposit to be 28 km³, within the 0.5 isopach contour. We note that had we just used the uncorrected data in Fig. 1a the volume estimate would only have been 23 km³.

The bulk density of the abyssal tephra was determined as 1.0 g cm⁻³. The density of the original magma was calculated at 2.2 g cm⁻³ using the method of Bottinga and Weill²² for the average glass composition at a temperature of 850 °C, taking into account the 10% phenocrysts in the original melt and assuming a water content of 3.0%. Thus the dense rock equivalent volume (DRE) is estimated at 13 km³. There is a substantial discrepancy between the DRE volume and the volume required to account for the caldera (60 km³). We stress, however, that our estimate is very much a minimum for several reasons. Firstly, the volume outside the 0.5 cm isopach may be substantial; furthermore, the volume north of the dispersal axis is unconstrained simply because of insufficient data coverage and the axis could well be further north, leading to increased volume estimates. Finally a considerable proportion of the magma is believed to have been erupted as pyroclastic flows⁶ which formed substantial accumulations of tephra transported by turbidity currents into the basins surrounding Santorini. The number of available cores and their poor penetration close to Santorini is insufficient to estimate the volume of pyroclastic flow material.

Effects of the eruption on the Minoan civilisation

The new data provide improved constraints on the thickness of tephra which could have fallen on Crete. Figure 1b illustrates that the freshly fallen thickness probably fell in the range 0–5 cm. In eastern Crete, where the majority of Late Minoan settlements occurred²³, the thickness must have been between 1 and 5 cm depending on the locality. Very much greater thicknesses or very much less would be very difficult to reconcile with the data, and present understanding of tephra fall-out patterns. Such tephra thicknesses would undoubtedly have been an inconvenience to the Minoan population and could have produced some minor agricultural damage which may have been moderately severe in the extreme northeastern part of the island. Thorarinsson¹⁴ has documented that greater than 10 cm of freshly fallen tephra resulted in the abandonment of farms in Icelandic eruptions. We note, however, that agricultural techniques in Minoan times may have been less able to cope with tephra-fall than in more recent times.

Our data suggests that the Minoan colonies on Rhodes and the south coast of Turkey may have suffered severe tephra-fall, whereas tephra-fall on Crete was probably insufficient in itself to cause a major decline of the Minoan Civilisation. We emphasise that our data does not, however, preclude a relationship between the eruption and the demise of the Minoan Civilisation. Present understanding of the direct effects (for example, tephra-fall) and indirect effects (for example, tsunamis, earthquakes and meteorological phenomena) of very large explosive eruptions is very limited, as eruptions of this magnitude are yet to be chronicled.

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Watkins died on 2 November, 1977. The other authors deeply regret this tragedy to the man who inspired and led the present work on the Eastern Mediterranean.

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Tumour-specific phenylalanine tRNA contains two supernumerary methylated bases

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Every malignant tumour examined contains aberrant tRNA methyltransferases and a few tRNAs which are absent from the normal tissue of origin. To determine whether tumour-specific tRNAs have different modifications from those in normal tissue, we purified the most frequently occurring tumour-specific isoaccepting tRNA from two malignant tissues. The isoaccepting phenylalanine tRNA from Novikoff hepatoma and Ehrlich ascites cells both contain two supernumerary methylated bases. One of these 1-methylguanine, is absent from the phenylalanine tRNA of normal rat, mouse, rabbit and calf liver. An increase in the levels of 5-methylcytidine and dihydrouridine was also detected.

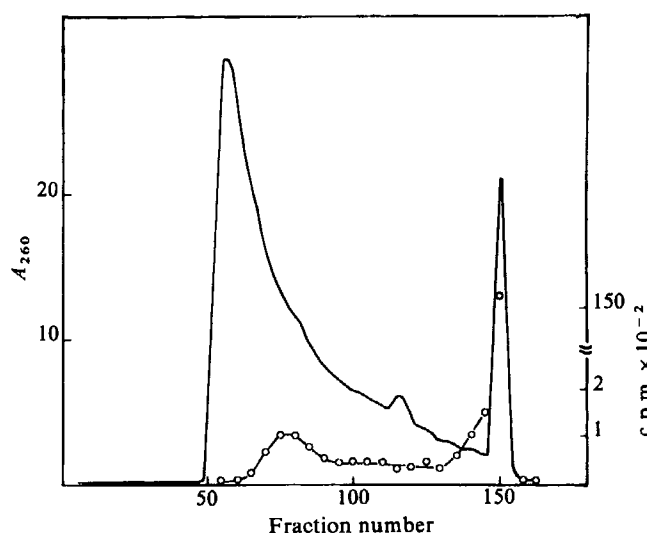
THE tRNA methyltransferases are aberrantly hyperactive in every malignant tumour examined and in each there are a few altered isoaccepting tRNAs which are absent from the tissue of origin¹. Whether the tumour-specific tRNAs differ in primary sequence or in modification has remained obscure. An analysis of the base sequence and of the modified bases in a pure, tumour-specific tRNA could establish the genesis of the altered tRNAs. If the primary sequence is found to be altered, it would imply that the tRNA originated either from a different or a mutated gene, or from error-prone transcription. If the primary sequence is unaltered, but some modifications are either absent or are supernumerary, then the tumour-specific tRNA is the product of aberrant modification.

There are conflicting reports in the literature about the modified base content of tRNAs in tumour tissue. Such confusion is not unexpected because until now, all the analyses have been performed on bulk tRNA and since most of the tRNAs in tumour tissue are identical to those of their normal counterparts, any changes in the few

tumour-specific tRNAs may be masked by the composition of the normal preponderant species.

Inspection of the literature reveals that the most frequently altered tRNA in tumour tissue is tRNA^{Phe}; therefore we undertook the purification of this tumour-specific tRNA from two different neoplasms, to compare

Fig. 1 BD-cellulose column chromatography of Novikoff hepatoma tRNA. The column (2×100 cm) was previously washed with 0.02 M sodium acetate buffer (pH 6.0) and 0.2 M NaCl. Novikoff hepatoma tRNA (10,000 A₂₆₀ units) was applied to the column. A linear gradient elution was carried out using 1 l of 0.02 M sodium acetate buffer (pH 6.0) and 0.5 M NaCl in the mixing chamber, and 1 l of 0.02 M sodium acetate buffer (pH 6.0) and 1.5 M NaCl in the reservoir. The column was washed finally with 0.02 M sodium acetate buffer (pH 6.0), 2 M NaCl and 20% v/v of ethyl alcohol. A volume of 10 ml of the effluent was collected per tube. —, Absorbance at 260 nm; ○, ³H-phenylalanine acceptor capacity.



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the base composition of it with that of pure tRNA^{Phe} from normal tissues.

Normal mammalian tRNA^{Phe} contains a highly modified hydrophobic base (O₂Y-Wye) (peroxywybutine) which is located adjacent to the 3' end of the anticodon^{2,3}. Consequently this tRNA has a unique behaviour on benzoylated DEAE-cellulose (BD-cellulose), since it is retained much longer than other tRNAs (ref. 3). Isolation of this tRNA is, therefore, relatively easy. The elution behaviour of tRNA^{Phe} from the tumours was found to differ from their normal counterpart, so a more elaborate purification procedure had to be devised.

Purification of tRNA^{Phe} from liver

Normal rat and mouse liver tRNA^{Phe} were eluted as a single peak from a BD-cellulose column at 2 M NaCl concentration containing 20% ethanol. Normal rat liver tRNA^{Phe} was further purified by fractionation on a RPC-5 column. A single peak of tRNA^{Phe} was observed and the

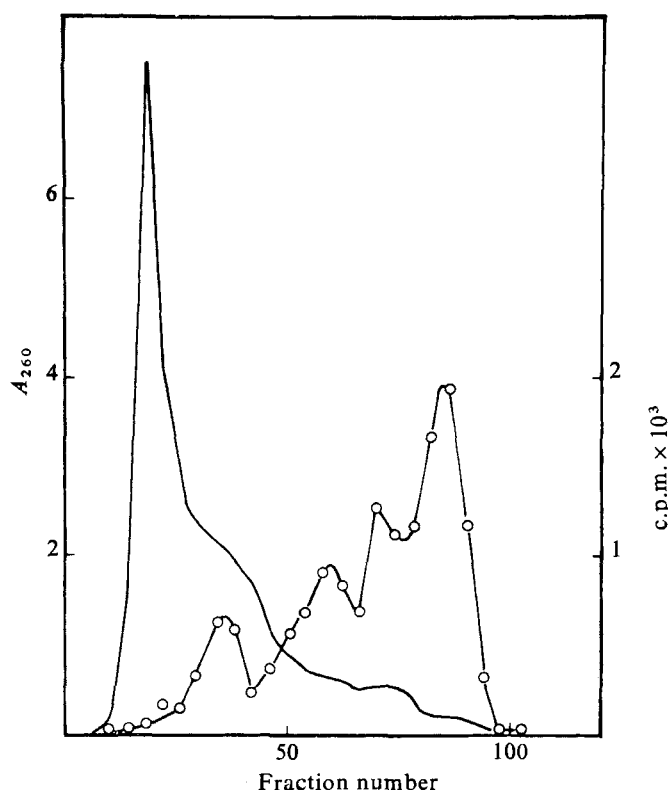


Fig. 2 Further purification of Novikoff hepatoma tRNA^{Phe} on a RPC-5 column. The column (1 × 50 cm) was previously washed with 0.01 M sodium acetate buffer (pH 4.7), 0.01 M MgCl₂, 2 mM β-mercaptoethanol and 0.4 M NaCl. The tRNA^{Phe}-rich region (750 *A*₂₆₀ units) obtained from a DEAE-Sephadex A-50 column (fractions 66–91, Fig. 1) was applied to the column. A linear gradient elution was carried out using 500 ml of 0.01 M sodium acetate buffer (pH 4.7), 0.01 M MgCl₂, 2 mM β-mercaptoethanol and 0.5 M NaCl in the mixing chamber, and 500 ml of 0.01 M sodium acetate buffer (pH 4.7), 0.01 M MgCl₂, 2 mM β-mercaptoethanol and 0.8 M NaCl in the reservoir. A volume of 5 ml of the effluent was collected per tube. —, Absorbance at 260 nm; ○, ³H-phenylalanine.

tRNA^{Phe} obtained from this column had an acceptance capacity of 1.36 nmol of phenylalanine per *A*₂₆₀ unit, indicating 82% purity. Purification of normal mouse liver tRNA^{Phe} was carried out using the same methods as on rat liver tRNA^{Phe}. Normal mouse liver tRNA^{Phe} purified this way accepted 1.51 nmol of phenylalanine per *A*₂₆₀ unit of tRNA, indicating 91% purity.

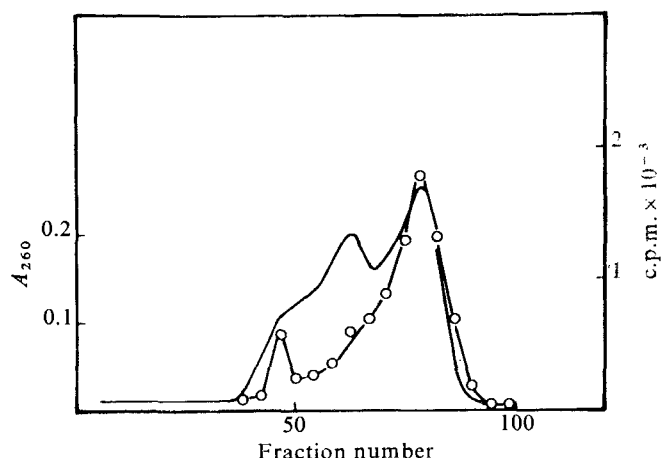


Fig. 3 Rechromatography of Novikoff hepatoma tRNA^{Phe} on a RPC-5 column. The conditions for chromatography were as in Fig. 2 except that 150 ml of the same acetate buffer in each chamber was used for elution. The tRNA^{Phe}-rich region (14 *A*₂₆₀ units) obtained from a RPC-5 column (fractions 76–95, Fig. 2) was applied to the column (0.6 × 30 cm). Each fraction contained 2 ml of the effluent. —, Absorbance at 260 nm; ○, ³H-phenylalanine acceptor capacity.

Purification of tumour-specific tRNA^{Phe} from two malignant tissues

Bulk tRNA was isolated from 1 kg of Novikoff hepatoma and was fractionated on a benzoylated DEAE-cellulose column by previously described methods^{4–6} (Fig. 1). The first minor peak, which was not detected on chromatography of normal rat liver tRNA, was further fractionated on a DEAE-Sephadex A-50 column. It eluted as a single peak and was further purified on a RPC-5 column. As seen in Fig. 2, the tRNA^{Phe} was resolved into at least three peaks. The last major tRNA^{Phe} (fractions 76–95) was rechromatographed on the same column (Fig. 3). Fractions 75–86 were pooled and precipitated by adding 0.1 vol 1 M

Fig. 4 Elution profile of phenylalanyl-tRNA of Novikoff hepatoma and normal rat liver on a RPC-5 column. ³H- or ¹⁴C-labelled phenylalanyl-tRNA was prepared by previously described methods⁷. Novikoff hepatoma aminoacyl-tRNA synthetase was used to charge the tRNA. The conditions for chromatography were as described previously⁷ except that a linear gradient of 0.5–0.85 M NaCl was used. *a*, ³H-Phenylalanyl-tRNA from unfractionated Novikoff hepatoma tRNA (○) and ¹⁴C-phenylalanyl-tRNA from unfractionated normal rat liver tRNA (●). *b*, ³H-phenylalanyl-tRNA^{Phe} purified from Novikoff hepatoma (—) and ¹⁴C-phenylalanyl-tRNA^{Phe} from unfractionated Novikoff hepatoma tRNA (○).

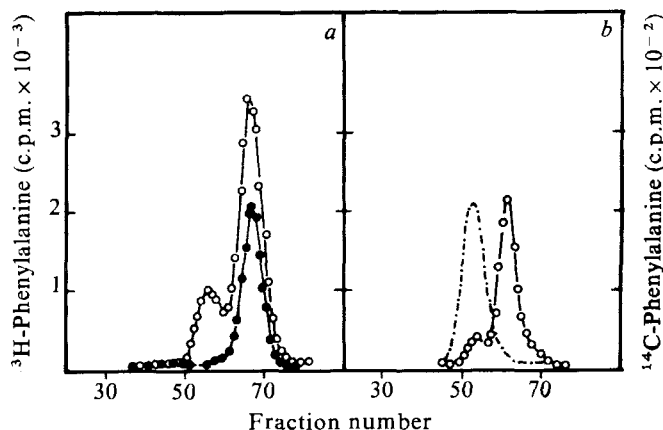


Table 1 Molar ratio of nucleosides of tRNA^{Phe}

Base	Mouse liver	Ehrlich ascites cells	Rat liver	Novikoff hepatoma	Rabbit* liver	Calf* liver
hu	2.7	4.0	3.1	4.2	2.2	3
m ¹ G	—	0.8	—	1.0	—	—
m ⁵ C	1.1	2.0	0.9	2.4	1	1
m ¹ A	2.1	2.1	2.0	2.0	2	2
T	0.6	0.5	0.8	0.6	0.7	0.5
m ₂ ² G	1.0	1.0	1.0	1.0	1	1
m ² G	1.1	1.1	1.1	1.2	1	1
Ψ	4.0	4.3	3.3	4.4	4	4
m ⁷ G	1.0	0.9	1.1	1.1	1	1
A	16.0	15.5	16.0	14.9	16	16
C	17.3	19.1	16.8	21.4	15	15
U	10.1	9.3	8.2	11.1	10	9.5
G	19.0	18.6	19.2	22.3	19	18

The tritium-labelled chromatographically separated trialcohols (as in Figs 5 and 6) were scraped from the plates, counted and their relative abundance calculated, assuming 1 mol of m₂²G per tRNA. That such an assumption is valid is indicated by the number of mol of nucleosides found: they range from 74 in tRNA^{Phe} from rat liver to 87 in Novikoff hepatoma. If two m₂²G were present, the number of mol of nucleosides would be twice those values.

* These data from ref. 9.

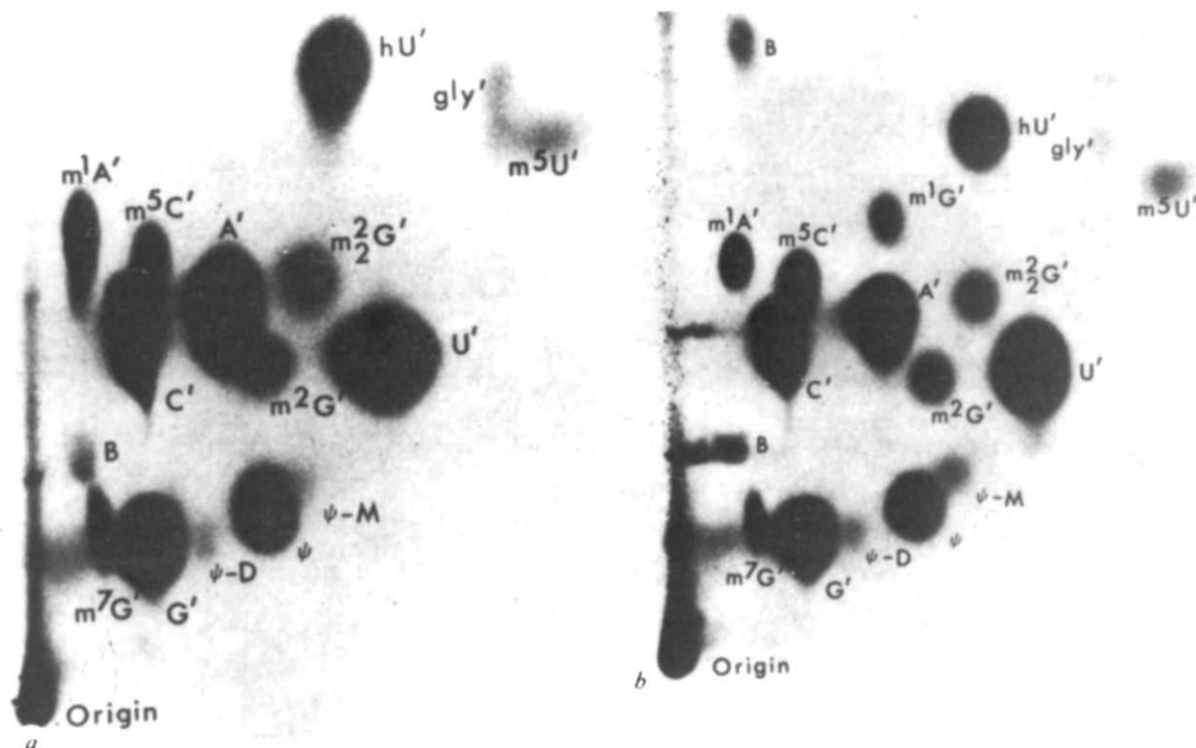
sodium acetate and 2.5 vol ethanol. Purified Novikoff hepatoma tRNA^{Phe} accepted 1.33 nmol phenylalanine per A₂₆₀ unit, indicating 80% purity.

Figure 4a shows the cochromatography on a reverse phase column (RPC-5) of phe-tRNA^{Phe} from unfractionated normal rat liver tRNA and from unfractionated Novikoff hepatoma tRNA. The cochromatography of the purified

phe-tRNA^{Phe} from Novikoff hepatoma (early eluting) with the unfractionated material is shown in Fig. 4b.

Bulk tRNA was isolated from 100 g of Ehrlich ascites cells, and a tumour-specific tRNA^{Phe} which had chromatographic behaviour identical to that of tumour-specific tRNA^{Phe} from Novikoff hepatoma was purified by the procedure described earlier. This preparation accepted

Fig. 5 Fluorograph of tRNA^{Phe} from *a*, normal rat liver and *b*, Novikoff hepatoma (0.2 A₂₆₀ unit). Purified tRNA^{Phe} was digested for 6 h at 37 °C with a reaction mixture containing 3 µg RNase A, 3 µg of snake venom phosphodiesterase, 2.4 µg of *E. coli* alkaline phosphatase, 0.01 M MgCl₂, 0.03 M bicine buffer (pH 8.0) in a final volume of 20 µl. After appropriate dilution, the enzymatic digests were subjected to periodate oxidation and reduction with ³H-KBH₄ to form the tritium-labelled nucleoside trialcohols. The tritium-labelled nucleoside derivatives were separated on cellulose thin layers in solvent A (Acetonitrile-4 N ammonia, 3.4:1, v/v) and solvent B (*t*-amyl alcohol-methylethylketone-acetonitrile-ethylacetate-water-formic acid, 4:2:1.5:2:1.5:0.18, v/v). The tritium-labelled compounds were detected on X-ray film by fluorography. The base composition was calculated as described by Randerath⁸. Abbreviations used: O₂Y-Wye, peroxywybutine; U', A', C', G', nucleoside trialcohols of uridine, adenosine, cytidine, and guanosine; m⁵U', m¹A', m⁵C', m²G', m₂²G', m⁷G', m¹G', nucleoside trialcohols of 5-methyluridine, 1-methyladenosine, 5-methylcytidine, 2-methylguanosine, 2,2-dimethylguanosine, 7-methylguanosine, and 1-methylguanosine; hU', Ψ', nucleoside trialcohols of dihydrouridine and pseudouridine; gly, glycerol; Ψ-D, Ψ-M, decomposition products derived from pseudouridine.



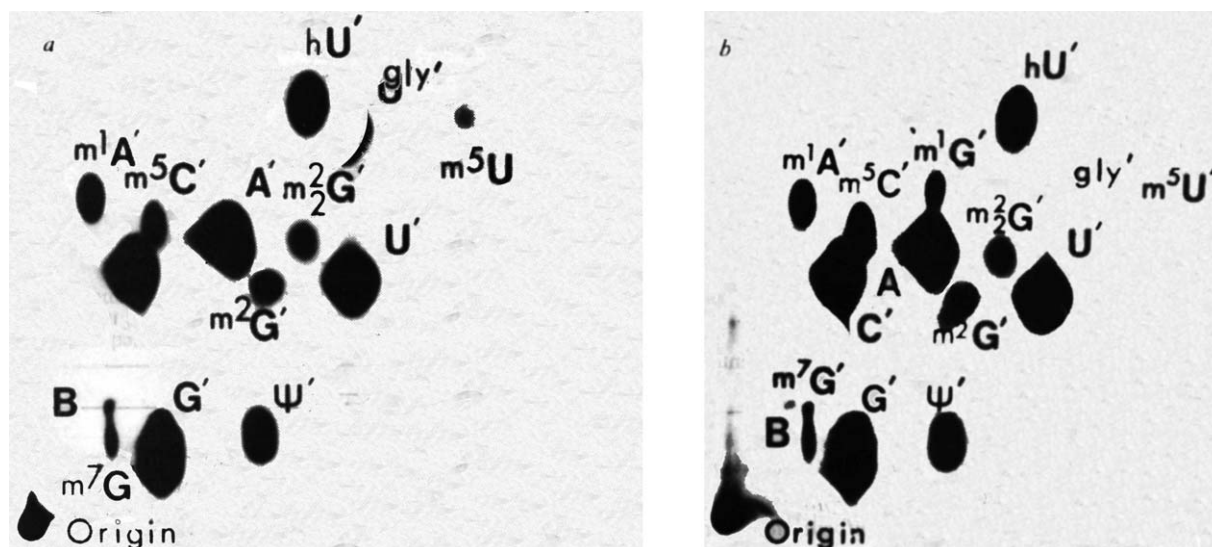


Fig. 6 Fluorograph of tRNA^{Phe} from *a*, normal mouse liver and *b*, Ehrlich ascites cells. The method and abbreviations are as used for Fig. 5.

1.48 nmol of phenylalanine per A_{260} unit of tRNA, indicating 89% purity.

Nucleoside composition of tRNA^{Phe} from tumour and normal tissues

Figures 5 and 6 show the nucleoside composition, determined by the method of Randerath⁸, of tRNA^{Phe} from normal rat liver (Fig. 5*a*), Novikoff hepatoma (Fig. 5*b*), normal mouse liver (Fig. 6*a*) and from Ehrlich ascites cells (Fig. 6*b*). The unique presence of 1-methyl guanosine in tRNA^{Phe} from both malignant tissues is noteworthy. This modification is also absent from tRNA^{Phe} from two other mammalian sources (Table 1).

Table 1 gives the molar ratios of the major and modified nucleosides to tRNA^{Phe} from four different normal mammalian sources and from the two malignant tissues. Two modified nucleosides, dihydrouridine and 5-methyl cytosine were found to be elevated by 1 mol in the tRNA^{Phe} from the malignant tissues; otherwise, the composition coincides to within experimental error with that of tRNA^{Phe} from other mammalian sources. The total number of nucleosides in tRNA^{Phe} from Novikoff hepatoma seems to be higher than in those from other sources. Whether this augmented structure is real will be resolved by sequence analysis which is under way.

Discussion

The findings reported here provide unequivocal evidence for aberrant modification of a tumour-specific tRNA, but this does not rule out the possibility of altered nucleoside sequence as well. The paucity of the supernumerary methylated bases is at first glance surprising, in view of the very high activity of the tRNA methyltransferases in malignant tumour tissues. Studies in our laboratory, however, have demonstrated a very high turnover rate of tRNAs of tumour tissue¹⁰. Whether excessively methylated tRNAs are rapidly eliminated remains to be determined.

Given the numerous and varied roles of minor species of tRNAs in other systems, it is difficult to predict what, if any, aberrant attributes these minor changes in modification may confer on the tumour cell. Ames *et al.* have shown that the lack of modification of two Us to ψ's in tRNA^{His} makes a profound impact on the economy of *Salmonella*. In the absence of the modification of tRNA^{His},

a mutant *Salmonella* is continuously derepressed for the production of nine enzymes in the histidine-synthesising pathway, even in the presence of an adequate supply of histidine¹¹. The consequent waste in energy and material for the cell is enormous.

A more recent implication of a role of methylation of tRNA for transcriptional control has been adduced by Freundlich and his coworkers¹², who found that if a methionine auxotroph of *S. typhimurium* was deprived of its essential amino acid, there were changes in the chromatographic profiles of tRNA^{Val}, tRNA^{Isoleu} and tRNA^{Leu}. Incomplete modifications by methylation were evidenced by the ability of the total population of tRNAs to serve as substrate for methylation by homologous enzymes. Concomitant to the changes in the tRNA elution profiles there was a 5–10-fold increase in the synthetic enzymes for isoleucine and valine even though an excess of these amino acids was available to the organisms.

The evidence for the loss of functional effectiveness in derepression by undermodified tRNAs is, at present, circumstantial. Perfection of appropriate systems of tRNA-dependent transcription is needed for unequivocal evidence for loss of regulatory capacity by undermodified tRNAs.

What functional capacity is endowed—or deprived—by aberrant modification of tRNA in tumour tissue is not known. As the reality of aberrant modification has now been established, however, a search for altered functions of such purified tRNAs can be undertaken with confidence.

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α -Helix-double helix interaction shown in the structure of a protamine-transfer RNA complex and a nucleoprotamine model

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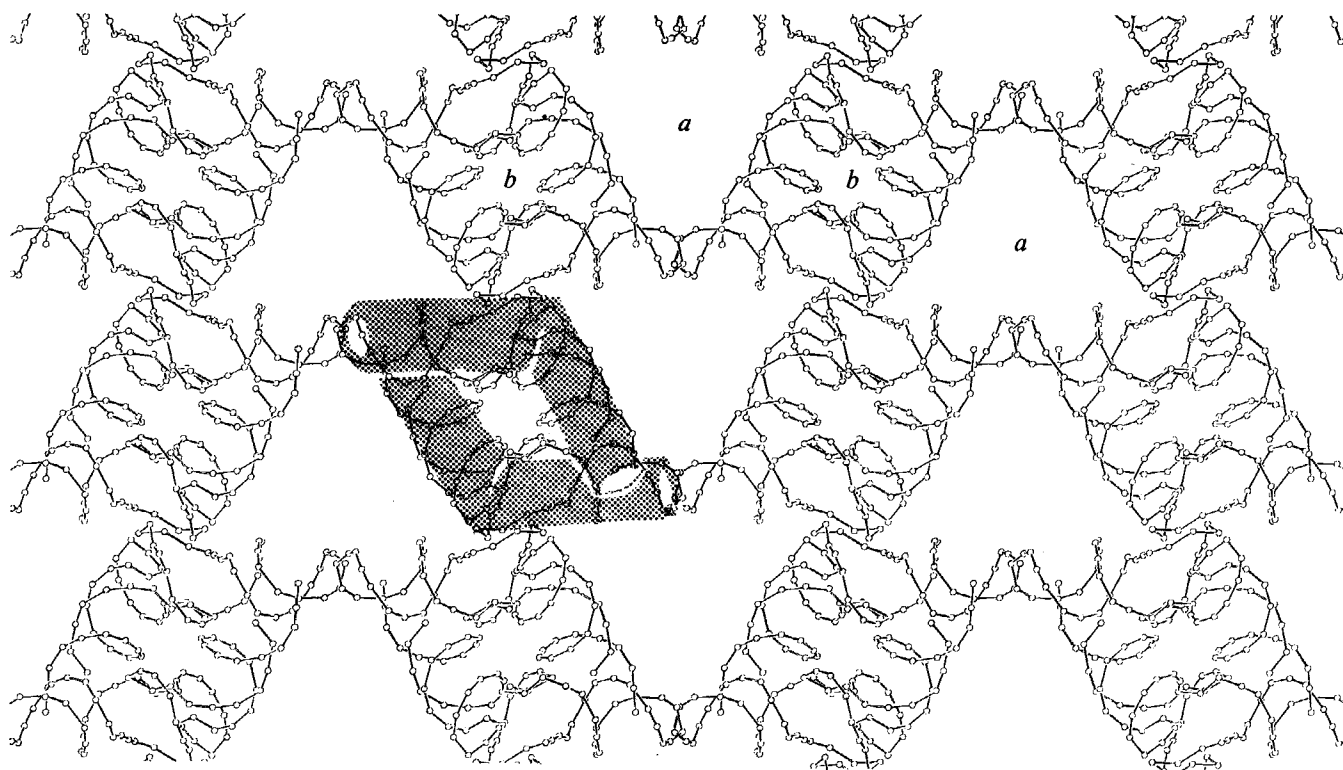
Single crystal X-ray diffraction and circular dichroism studies of protamine binding to a tRNA suggest that the protamine molecule changes its conformation from a random coil to a structure containing a helices on binding to tRNA, and that α -helical segment(s) of protamine bind approximately along a shallow groove of a double-helical portion of tRNA. Based on these observations, a structural model for nucleoprotamine is proposed.

PROTAMINES are extremely basic, small proteins of molecular weights of about 4,200. They are found tightly associated with DNAs in fish spermatozoa. Nearly two-thirds of the amino acid residues in protamines are basic, and these basic residues are usually found clustered, four or five in a sequence. When protamine messenger RNA (mRNA) reaches the cytoplasm, the residues are translated by diribosomes. The newly synthesised protamines are phosphorylated by ATP in cytoplasm,

catalysed by protamine kinase on serine residues. The phosphorylated protamines are transferred into the cell nucleus and then into chromatin, where they replace the histones and bind to DNA. During the maturation of the sperm heads, the protamine is dephosphorylated, the DNA becomes more condensed, and all transcription stops.

Much research has been directed to find the mode of binding between protamine and double-helical DNA by X-ray diffraction methods¹⁻³, spectroscopic techniques^{4,5}, electron microscopy⁶ and chemical modification⁷. The most widely accepted view at present is that protamine exists in an extended chain which wraps around double-helical DNA, covering the narrow groove along the DNA double helix^{1,2}, or sometimes crossing over to a neighbouring DNA double helix^{6,8}, with the positively charged basic side chains of protamine neutralising negatively charged phosphates of the polynucleotide chain. In a less specific model⁹, hexagonally arranged parallel rods of double-helical DNA are held together loosely by a network of protamines.

Fig. 1 Packing diagram of yeast phenylalanine tRNA in an orthorhombic crystalline form (P_{21221}) viewed along the crystallographic axis a . Two L-shaped tRNA molecules at an angle are shown shaded, and two types of channels are indicated. The large channels (a) have a diameter of $> 40 \text{ \AA}$, and the small channels (b) have a diameter of approximately $15 \text{ \AA}$. This figure represents the thickness of the unit cell along the a axis. In the crystal, this packing is repeated indefinitely in all three directions, all exactly registered. In this crystalline form, the volume occupied by tRNA is approximately 25%, and that for the buffer is approximately 75%.



To understand the interactions between protamines and nucleic acids in more detail, we investigated crystallographically the interactions between protamines and transfer RNA (tRNA) primarily because tRNA has been crystallised in several suitable single crystalline forms, whereas DNA has not.

Yeast phenylalanine tRNA crystallises into an orthorhombic lattice, in which 75% of the total volume is occupied by buffer solution. There are two kinds of channels in this crystal form (Fig. 1). The large channel, *a*, is over 40 Å in diameter and the small channel, *b*, is about 15 Å in diameter. Thus, this crystal form provides an open crystalline matrix into which various molecules can be soaked, to study their interaction with tRNA by single crystal X-ray diffraction techniques. Since a large portion of tRNA structure is double helical, this system can be considered for studies involving double-helical RNA in general. We report here the crystal structure of a protamine-tRNA complex at 5.4 Å resolution. Based on the information obtained from the structure and model building, a structural model for nucleoprotamine is proposed.

Materials and methods

Single crystals of transfer RNA were grown in a buffer containing 40 mM MgCl₂, 40 mM Na cacodylate, 3 mM spermine hydrochloride at pH 6.0 (ref. 10). Yeast phenylalanine tRNA was purchased from Boehringer-Mannheim, and protamine sulphate from salmon sperm was purchased from Sigma. Protamine sulphate was dissolved in the same buffer solution as the tRNA crystallisation buffer, and then added to a small depression containing tRNA crystals to yield a protamine-tRNA molar ratio of 4:1. After several days, the soaked crystals were sealed in glass capillaries and X-ray data were collected on an automatic four-circle diffractometer.

The sizes of crystals used for collection of X-ray data were about 100 × 150 × 500 μm. The soaked crystals had the same space group symmetry, and the cell dimensions changed less than 0.4%. Five steps per reflection were measured and individually fitted to a Gaussian curve to estimate integrated intensities for each reflection. A total of 1,253 reflections was collected up to 5.4 Å resolution, and of these, 1,011 had intensities significantly (greater than 4σ: where σ is an estimated standard deviation of each intensity) above the background. This data set was scaled to that obtained from a native tRNA crystal (which did not contain any protamine) as a function of resolution. The discrepancy factor (*R* factor) between these two data sets was 8% for the structure factor amplitudes. A difference electron density map was prepared, using the differences of amplitudes between these two data sets and the phases calculated from the atomic coordinates of the yeast tRNA^{Phe} structure in an orthorhombic lattice. The three-dimensional structure of this tRNA has been determined¹¹ and refined by a structure factor least-squares method¹².

Eleven residues from the C terminus of a salmon sperm protamine, Salmine AI, were folded to generate an α helix and then fitted to the difference electron density map obtained as described above, using an interactive computer graphics system at the University of North Carolina, Chapel Hill. After a portion of the α helix was fitted approximately into the electron density, it was further refined by treating the α helix as a rigid body. Three rotational and three translational parameters, as well as the occupancy and the overall temperature factor, of the α helix were refined by minimising the difference between calculated structure factors and the observed structure factors using a least-squares method¹³.

Circular dichroism (CD) measurements were made on a Dichrographe III of Jobin-Yvon using a quartz cell with path-length of 1 mm. The stock solutions for the CD measurements were: a buffer solution containing 10 mM MgCl₂, 10 mM Na cacodylate and 1 mM spermine·4HCl at pH 6.0; a tRNA solution containing 5.3 mg ml⁻¹ of yeast tRNA^{Phe} in the buffer described above; the protamine solution containing 12.5 mg ml⁻¹ salmon protamine sulphate in the same buffer. CD profiles were recorded for each of these solutions, and for

mixtures with protamine-tRNA molar ratios ranging from 0:1 to 16:1.

Structure of a protamine-tRNA complex

The results of the CD measurements are shown in Fig. 2. As protamine was added to the tRNA solution, we observed an increase of precipitation, and a decrease of tRNA in solution, indicated by the reduction in the magnitude of θ while the curve shape remained the same. When the molar ratio of protamine to tRNA reached 4:1, practically all tRNA and protamine in the solution was precipitated (Fig. 2*f*). This molar ratio corresponds to a stoichiometry of 1.1 positive charges of protamine per 1 negative charge of tRNA, if one assumes that salmon sperm protamine AI is representative of all salmon sperm protamines. At present, this is the only protamine from salmon sperm that has been sequenced¹³, and it contains 32 residues of amino acids of the following sequence:

PRO ARG ARG ARG ARG SER SER SER ARG
PRO VAL ARG ARG ARG ARG ARG PRO
ARG VAL SER ARG ARG ARG ARG ARG
ARG GLY GLY ARG ARG ARG ARG

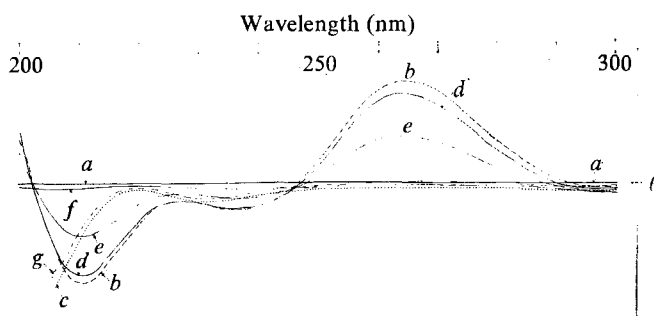


Fig. 2 Circular dichroism spectra of complex formation between salmon sperm protamine and yeast phenylalanine tRNA. The molar ellipticity θ is on one axis and the wavelength (nm) on the other. The θ value of curve *b* at 265 nm is 1.895×10^4 degrees $\text{cm}^2 \text{dmol}^{-1}$. The measurements were made for the wavelengths between 300 nm and 205 nm. *a*, A buffer solution containing 10 mM MgCl₂, 10 mM Na cacodylate and 1 mM spermine·4HCl with pH adjusted to 6.0; *b*, tRNA solution containing 1.0 mg ml⁻¹ of yeast tRNA^{Phe} in the buffer solution described above; *c*, the protamine solution containing 4.0 mg ml⁻¹ salmon protamine sulphate in the same buffer; *d*, 12.5 mg ml⁻¹ protamine stock solution was added to the tRNA solution described for *b* to give a molar ratio of protamine-tRNA of 0.25:1; *e*, molar ratio of 1:1; *f*, molar ratio of 4:1; *g*, molar ratio of 16:1.

The difference electron density map revealed only one kind of long, partially segmented electron density peak, running zig-zag along the small channel (channel *b* in Fig. 1) formed by tRNA molecules in crystalline lattice as shown in Fig. 3. This was the only peak present when contoured at the level of 5σ where σ represents root mean square electron densities in the difference electron density map. There were no significant negative peaks either. Each electron density segment is approximately cylindrical and corresponds in size to an α helix of about nine or ten amino acids (see Fig. 3C). Each segment is in contact, on a shallow (minor) groove side, with both strands of one double helix from a tRNA molecule (see Fig. 4) and one strand each of two other double helices from a neighbouring tRNA molecule.

When the α-helical portion of the structure was refined by a least-squares method, the occupancy and the thermal parameter were 20% and 90 Å² respectively. Since the asymmetric portion of the electron density can accommodate only about nine or ten of 32 amino acid residues, we assume that a part or all of the protamine molecule is partially disordered along the chain of electron density and that the kinking of the electron densities probably represents the kinking of the α helix at sites prior to

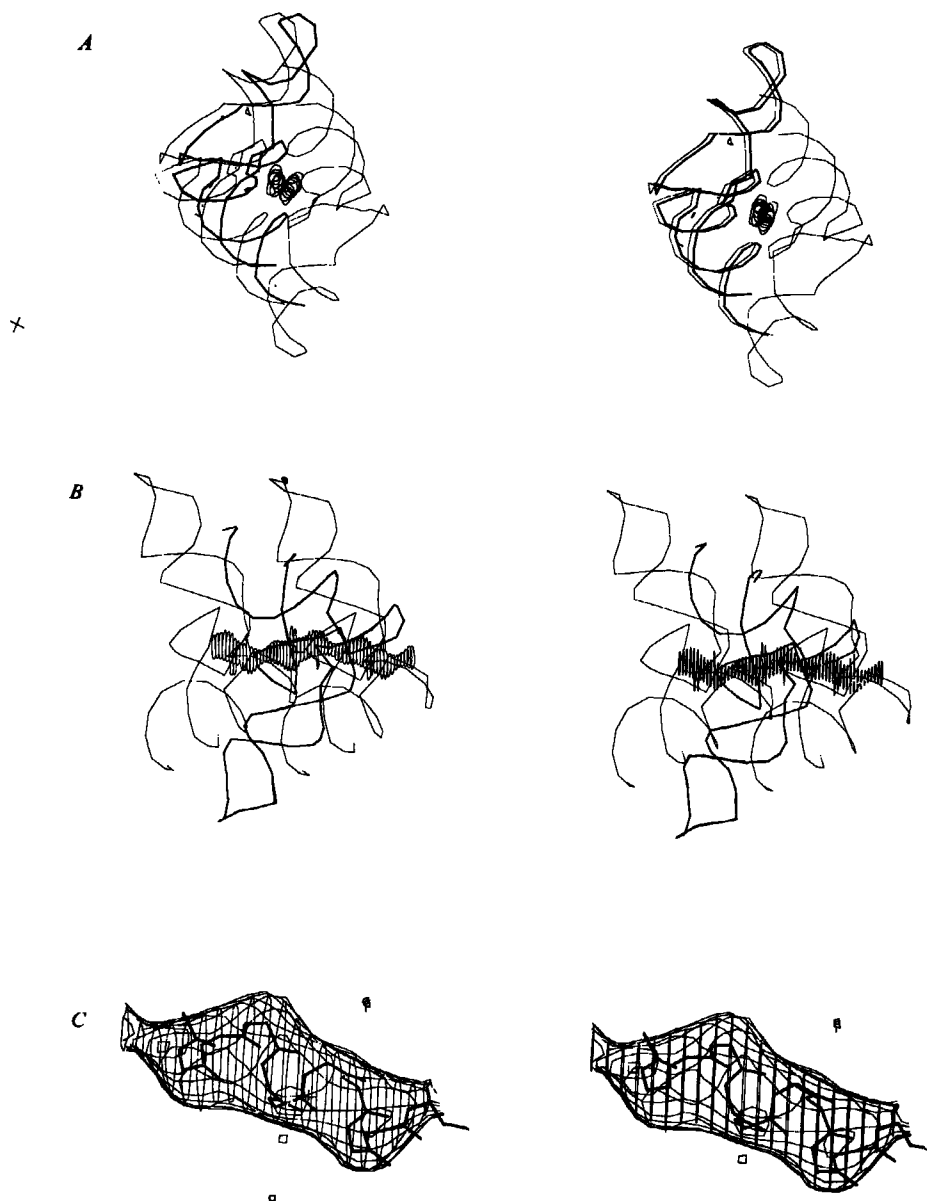


Fig. 3 The difference electron density map between the crystal of protamine-yeast tRNA1/1 and the native crystal of yeast tRNA1/1. The contouring level was chosen to show, primarily, the major feature of the difference electron density. The backbone structure of the tRNA is superimposed to show the relative position of the electron density peaks in the crystalline lattice. *A*, viewed approximately along the crystallographic axis *a* (for clarity, only two cylindrical segments are shown). *B*, Three cylindrical segments, viewed along the crystallographic axis *b*. The channels are running approximately along the crystallographic axis *a*. *C*, The fitting of a unique asymmetric portion of the difference electron density map with an α -helix ten residues long.

two internal prolines and a pair of glycines in the amino acid sequence (see Fig. 5 and below).

Conclusions and discussions

On the basis of our results from single crystal X-ray diffraction and CD studies, we make the following conclusions.

The protamine molecule changes its conformation from a random coil structure to a structure containing one or more α -helical segments in the presence of tRNA and possibly other nucleic acids. This is based on the observation that protamine in solution, in the absence of tRNA, gives a CD spectrum typical of a mostly random coil (see curve *c* of Fig. 2). This observation is consistent with the earlier observations from deuterium exchange and spectroscopic experiments^{4,14}. In the presence of tRNA, however, the protamine forms a complex with tRNA and precipitates. The crystal structure of the protamine-tRNA complex suggest that at least a portion of the protamine which is in contact with tRNA is in an α -helical conformation.

In a protamine-tRNA complex, the stoichiometry of positive charges of protamine to negative charges of tRNA is approximately 1:1. This is based on the observation from the CD study which shows that when the molar ratio of protamine to tRNA is 4:1, all the tRNA and protamine have been complexed and precipitated and no appreciable tRNA or protamine remains in

solution. At such a molar ratio, the stoichiometry between positive charge from protamine and negative charges from tRNA is approximately 1 to 1. This charge stoichiometry is the same as that observed for protamine-DNA complexes (for review, see ref. 8).

The protamine molecule binds to tRNA nonspecifically. As mentioned earlier, the yeast phenylalanine tRNA in the orthorhombic crystal form occupies only 25% of the crystal volume, with the remainder being buffer solution. tRNA molecules in this crystal form make a small number of contacts which barely maintain the crystalline lattice, thus most of the molecular surface of tRNA is exposed to buffer solution and should therefore be available for protamine binding. In fact, the absorbance measurement at 202 nm of the mother liquor in equilibrium with tRNA crystals showed that more than 99.3% of the protamine added was bound in the tRNA crystals. The difference electron density map, however, shows that the significant peaks are found only in the small channel *b* of Fig. 1. Since the single crystal X-ray diffraction studies can reveal only the ordered or partially ordered portion of the protamine structure, this crystallographic observation can be interpreted as follows: The protamine binds to all available parts of tRNA. But they bind with no site specificity and, therefore, they are not ordered or partially ordered in a crystalline lattice. In channel *b*, however, which is relatively narrow and restricted spatially by

the tRNA molecules nearby, the protamine molecules probably have much less freedom in binding and thus are partially ordered, and therefore 'visible' by single crystal X-ray diffraction techniques.

Protamine molecules, or the α -helical portion of protamine molecules, can stabilise not only an individual double helix, but also two or three adjacent double helices, thus helping the close packaging of double-helical RNA and possible DNA. This is based on the following observations: at a 4:1 molar ratio of protamine-tRNA, protamine helps to aggregate and precipitate tRNAs; in the crystal, the α -helical portion of protamine is sandwiched between a double-helical portion of tRNA from one molecule and one strand each from two more double-helical portions of a neighbouring tRNA molecule (Fig. 3A). Within this contact region, the distance between the α helix of the protamine segment and the phosphate groups of tRNA suggests that the guanidinium groups of extended arginine side chains can hydrogen bond to and, at the same time, neutralise, the negative charges of phosphates of tRNA. In the crystal, the α -helical segment was seen to bind to the shallow (minor) groove but not to the deep groove of the double helical portions of tRNA (Fig. 4), probably because only the shallow grooves of the T stem and acceptor stem are exposed to channel B.

From these conclusions, some general comments may be made about the possible mode of interaction between protamine and double-helical RNA or DNA. Given that protamine changes its conformation from a random coil to an α -helical structure in the presence of double-helical RNA or DNA, then Salmine AI protamine can be described, for example, as a structure with four segments of α helices with an average of about eight residues each, joined by three partially flexible joints, as shown schematically in Fig. 5. These joints are at residues 9, 16 and 26, all followed by well known helix-breaking residues, proline and glycine¹⁵. This prediction also follows from the consideration that, since each segment contains similar amino acids, if one segment assumes α -helical conformation in certain conditions, the others are also likely to assume the same conformations in the same condition, yielding four α -helical segments.

Nucleoprotamine model

The amino acid sequences of several fish sperm protamines have been determined (for a convenient compilation, see ref. 16). All of these sequences have the following aspects in common (see Fig. 6): they are 31–34 residues long; each begins with proline or alanine, followed by eight to ten residues, of which all except three are basic; and then another proline residue followed by five or six residues, all except one being basic; then another proline or tyrosine, followed by 13–17 residues,

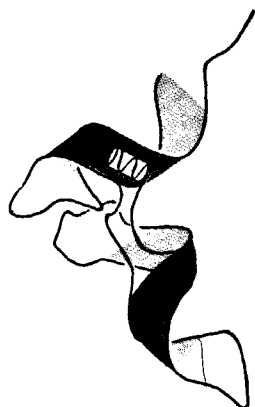


Fig. 4 Schematic drawing showing the location of an α -helical segment bound to the shallow groove of the T stem of yeast phenylalanine tRNA. The α -helical segment is shown as a cylinder. The shallow grooves in the tRNA structure are shaded.

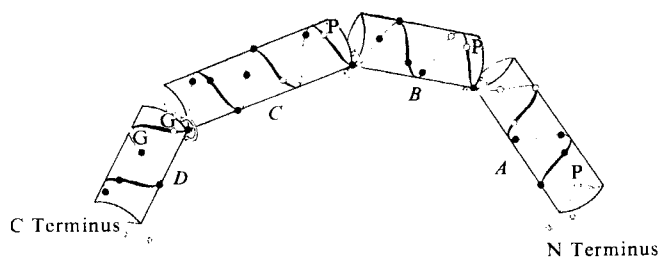


Fig. 5 Diagrammatic representation of Salmine AI protamine structure. This protamine is assumed to be composed of four α -helical segments joined by three partially flexible joints at residues just prior to two internal prolines and one joint before a pair of glycine residues. Arginines are shown as dark circles; and P and G stand for proline and glycine respectively.

all except three to six being basic amino acids. Thus, one can consider the general structure of protamine as composed of at least three segments, with two middle joints being a proline, tyrosine, or alanine residue. Even the last segment can be subdivided, in most cases, into two with a joint before a pair of glycines or valines.

Based on the observations from the crystal structure of the protamine-tRNA complex described here, we propose that all protamines have a structure composed of three or four α -helical domains connected by two or three flexible joints as suggested in Fig. 6 and shown schematically in Fig. 5 for Salmine AI protamine. Each α -helical domain contains four or more consecutive arginine residues, and can lie approximately along either groove of double-helical DNA. Two arginines facing the groove can neutralise and hydrogen bond to two negatively charged phosphates across a groove of one double helix. At the same time, the remaining arginines in the same α -helical domain can hydrogen bond to and neutralise the negatively charged phosphates of the neighbouring double helices, thus 'condensing' many double helical DNAs.

Although the backbone of an α helix does not have a structural complementarity to double-stranded nucleic acids as was noted for a β ribbon¹⁷, two nearby residues with long side chains like arginine and lysine in an α helix can place both basic groups about 10 Å–16 Å apart, depending on the side-chain conformation, just suitable to span across either groove of a double-stranded DNA or RNA.

A schematic drawing of the nucleoprotamine model is shown in Fig. 7 and described below. Model building suggests that the α -helical domains are more likely to lie on the major groove of double-helical DNA, although the minor groove cannot be ruled out. Two (or three when the arginine tract is longer than four) arginines of each domain neutralise and hydrogen bond to two (or three) phosphates across a major groove and the remaining arginines do the same to the phosphates of neighbouring double helix or helices. Each domain is thus binding or 'cross-linking' two or more double helices together, thereby causing a heavy condensation of DNA double helices.

Since the protamine α helices lie mostly on the major groove in this model, it is consistent with the observation in X-ray diffraction pattern of nucleoprotamine fibres, where the first layer-line intensities are stronger compared with the second layer-line^{1,2}. The di- or trivalent binding property of the α -helical segments of protamine can explain the electron microscopic observation on reconstituted nucleoprotamine⁶, where double-helical DNAs were shown to form a network. This also explains the observation that nucleoprotamine fibres do not swell as much as DNA on exposure to high humidity¹. Such cross-linking ability is likely to stabilise superhelical structure of DNA also. Although α -helical domains of protamine lie on the major groove of DNA double helix, the surface of the groove is essentially exposed to the solvent, because of the large spacing between the axis of the α helix and the groove surface caused by the long reach of arginine side chains (shown

SALMINE AI	P + + + + S S S +	P V + + + + +	P + V S + + + + +	G G + + + +
IRIDINE I(A)	P + + + + S S S +	P V + + + + +	P + + V S + + + + +	G G + + + +
IRIDINE I(B)	P + + + + + S S S +	P I + + + + +	P + + V S + + + + +	G G + + + +
IRIDINE II	P + + + + S S S +	P V + + + +	A + + V S + + + + +	G G + + + +
THYNNIN Y1	P + + + + E A S +	P V + + + + +	Y + + S T A A + + + + +	V V + + + +
THYNNIN Y2	P + + + + Q A S +	P V + + + + +	Y + + S T A A + + + + +	V V + + + +
THYNNIN Z1	P + + + + + S S +	P V + + + + +	Y + + S T V A + + + + +	V V + + + +
THYNNIN Z2	P + + + + + S S +	P V + + + + +	Y + + S T A A + + + + +	V V + + + +
CLUPINE Z	P + + + + S + + A S +	P V + + + +	P + + V S + + + +	A + + + +
CLUPINE YI	A + + + + S S S +	P I + + + +	P + + + T T + + + +	A G + + + +
CLUPINE YII	P + + + T + + A S +	P V + + + +	P + + V S + + + +	A + + + +



Fig. 6 All the known sequences of protamines¹⁶ are arranged to show four proposed domains. Abbreviations: A, alanine; E, glutamic acid; G, glycine; I, isoleucine; P, proline; Q, glutamine; S, serine; T, threonine; V, valine; Y, tyrosine; +, arginine.

schematically in the inset of Fig. 7). This aspect of the model is consistent with the results that dimethyl sulphate can methylate the N-7 atom of guanine in the major groove and the N-3 atom of adenine in the minor groove of the DNA double helix with equal relative efficiency but with small retardation on the major groove side⁷ at low temperature.

Functional implications of the model

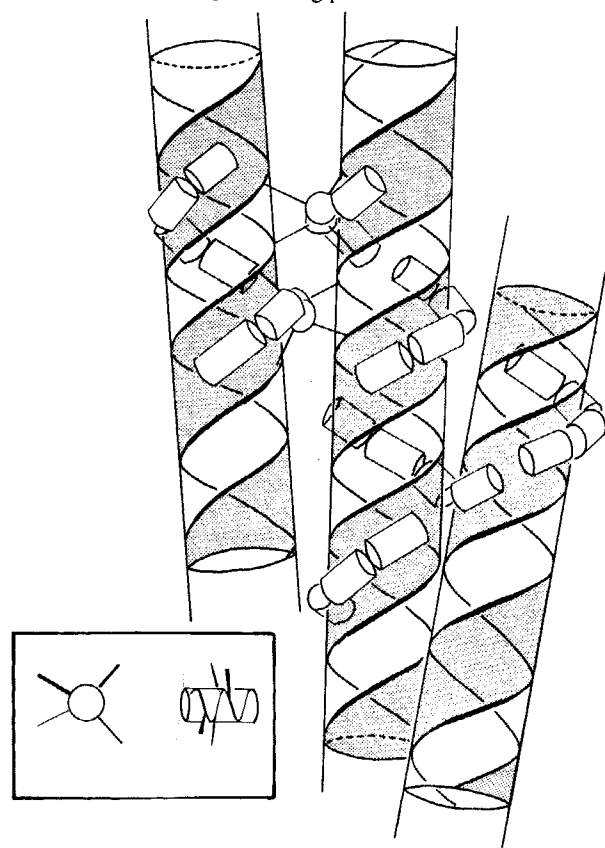
The protamine structure proposed here has several advantages as a DNA-condensing agent: The α -helical domains have structural rigidity and just sufficient length to stabilise DNA double helix-double helix cross-linking or 'condensation'; also, the joints between the α -helical domains provide flexibility in the orientation of the domains, so that two DNA double helices in variety of orientation can be brought together. Such extensive DNA condensation is probably the major reason for the inhibition of DNA-dependent RNA synthesis by protamines¹⁸.

The newly synthesised protamines are usually phosphorylated at serine residues¹⁹. The model proposed here suggests that such modification on protamines will interfere with the cross-linking of two adjacent DNA double helices. The following sequence of events can be imagined: The phosphorylation of protamines is probably necessary so that the modified protamines can efficiently displace histones and prevent DNA condensation (which will complicate the process). Once histone displacement is almost complete, dephosphorylation starts and so does the concomitant DNA condensation as a necessary step in the sperm maturation process.

The proposed model can explain several experimental observations and suggests some functional implications; however, the reason for the existence of multiple components in a protamine is not obvious from the model. It is likely that the similar interaction between two well defined structural elements, the α helix of protein and double helix of nucleic acids, may be found in other complexes between proteins and double-helical nucleic acids, such as in nucleosomes.

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Fig. 7 Schematic drawing to show parts of nucleoprotamine structure. Protamine molecules are represented by several connected cylinders; each corresponds to an α -helical segment. They wrap around the major groove (shaded) of the DNA double helix shown as a long large cylinder. Inset, an α -helical segment with four consecutive arginine residues (represented as four rods radiating out from the cylinder) is shown in two-orthogonal orientations. Two types of possible cross-linking are shown. Between the left and middle DNA double helices, there are two α -helical segments with arginine side chains shown as straight lines. Each segment forms two contacts across a major groove of one DNA double helix and two across a minor groove of the other DNA double helix. Between the middle and the right DNA double helices, three α -helical segments from one protamine molecule are in contact on the major groove of the middle DNA double helix and the fourth segment on the major groove of the right DNA double helix. There are many other types of cross-linking possible.



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letters to nature

X-ray lines and magnetic field of Her X-1

THE report¹ of a possible detection of an X-ray emission line with energy about 53 keV from the binary X-ray source Her X-1 created great excitement. Interpreting the line as arising from non-relativistic electron cyclotron emission at the surface of an accreting neutron star member of the binary star system, Trumper *et al.*¹ deduce a neutron star surface magnetic field of 4.6×10^{12} G. If the line is real (of cosmic origin and not due to background effects), significant (not due to a statistical fluctuation) and correctly interpreted as arising from non-relativistic cyclotron emission) it could provide the first direct and quantitative measure of the magnetic field of a neutron star, and be direct support for our ideas on the formation of neutron stars and the role of magnetic fields in the radiation mechanisms of pulsars and binary X-ray sources. Furthermore, it would conclusively rule out the possibility that Her X-1 contains a rotating or pulsating white dwarf (which, for dynamical stability requires $B \ll G^{1/2} MR^{-2} \approx 10^{12}$ G for $M \approx M_{\odot}$ and $R = R_{WD} \approx 10^9$ cm).

Coe *et al.*² reported the detection of a line at 64 ± 6 keV in the Her X-1 X-ray spectrum from observations made with the Ariel 5 satellite. The energy of their line is consistent, within observational uncertainties, with the line energy reported by Trumper *et al.*¹ (53 ± 8 keV). In light of these two reports, we have re-examined the UCSD OSO-7 observations³ of Her X-1 made in 1971 and 1972. As can be seen in Fig. 1, these data from May to June 1972 contain a large deviation from the blackbody best fit at about 25 keV. This feature seems to be about as significant (or insignificant) as that reported by Coe *et al.*² No X-ray flux was detected at energies above about 50 keV for this source. The > 50 keV data were, therefore, combined to form the upper limit shown in Fig. 1. As can be seen, the best fit continuum falls steeply and we could set no meaningful upper limit to a 60 keV line.

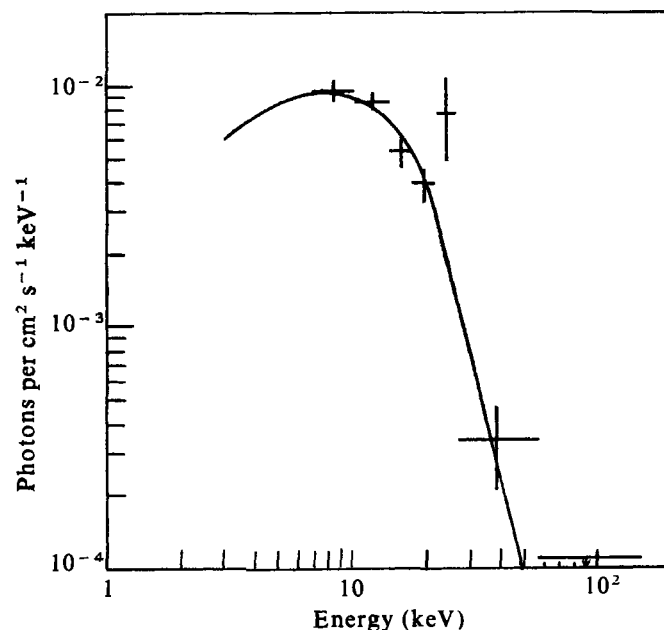
The observations of Her X-1 were made with the UCSD OSO-7 NaI (TI) 64 cm² 1 cm thick actively collimated detector. The spin rate of the satellite was 2 s per rev. This allowed continuous measurements of both the X-ray source and detector plus sky background^{3,4}. The 29 May to 1 June Her X-1 spectrum derived shows a feature at about 25 keV. If this feature were due to a line from Her X-1 itself, we estimate the intensity of the line as $2.4 \pm 1.2 \times 10^{-2}$ photons cm⁻² s⁻¹ centred at about 25 keV. A cosmic X-ray line would produce a deviation in at least one other energy channel (since one channel is about 4 keV wide), and by a judicious choice of continuum, a cosmic X-ray line is consistent with the data. But, there are alternative interpretations of such a feature. We discuss the two most likely ones below.

The UCSD OSO-7 instrument was subjected to bombardment by many low energy protons and neutrons during its passages

through the South Atlantic Anomaly. As a result, several features were present in the detector background, including an apparent line at about 30 keV, as well as a line at about 60 keV as shown in Fig. 2. These features are due to conversion of the iodine in the NaI (TI) crystal to radioactive nuclei. Effects such as this have been discussed extensively^{5–9}. In the UCSD OSO-7 experiment, the detector background 25 keV feature is comparable in intensity to the derived 25 keV flux from Her X-1. We tried to eliminate the feature from the Her X-1 data by using only the results taken a long time after the last South Atlantic Anomaly passage of the satellite. The feature remains as shown in Fig. 1. We estimate that an error in the background subtraction could have caused no more than a 10% error in the derived 25 keV flux from Her X-1. The one outstanding feature, however, in the OSO-7 Her X-1 data occurs at just the energy of one of the X-ray detector's background lines.

Trumper *et al.*¹ do not report a line at ~ 25 keV. If the OSO-7 Her X-1 feature were cosmic in origin, it might be variable, or Trumper *et al.* and Coe *et al.* might not have had the sensitivity to

Fig. 1 Average spectrum of Her X-1 from the UCSD OSO-7 cosmic X-ray detector³. Observations from 29 May–1 June 1972. The solid line fit is a minimum blackbody fit giving $T = 5.6 \times 10^7$ K.



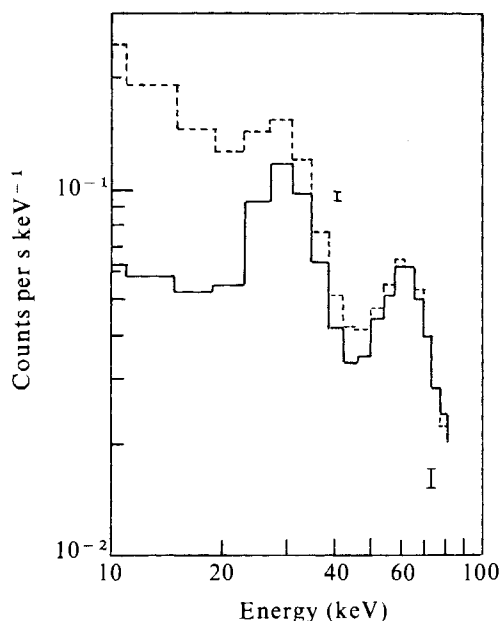


Fig. 2 Energy deposition spectrum for the UCSD OSO-7 cosmic X-ray detector. The solid line is derived from Earth-looking portions of the satellite orbit. The dashed line is derived from sky looking data excluding strong cosmic X-ray sources⁸.

an X-ray line of the OSO-7 instrument near 25 keV. The lowest energy that the Coe *et al.*² instrument could detect is about 25 keV, which might not have been low enough to be relevant to this report.

Another explanation that cannot be excluded is that the 25 keV Her X-1 feature is simply a statistical fluctuation. This is because: (1) the data point is only about 2σ above the continuum flux and (2) *a priori*, we do not know where to expect the line, and therefore finding a line in any one of five channels is five times as likely as deduced on the basis of simple Poisson statistics. This makes the present 'detection' only about 1.5σ . But, the same factor applied to the results of Trumper *et al.*¹ for example, also reduces the significance of the results from their two detectors from 6.2 and 4 σ to about 5 σ and 3 σ respectively.

Adding the statistical uncertainty to the possibility of background contamination, it is unlikely that the UCSD OSO-7 keV feature in the Her X-1 spectrum is of cosmic origin. On the other hand, we cannot exclude such a possibility. We shall therefore briefly discuss the consequences of assuming the line is real and arises from cyclotron emission from Her X-1.

Even if one or all of the line features discussed above are of cosmic origin and arise from cyclotron radiation from electrons moving in the strong magnetic field of a neutron star, many uncertainties arise in trying to make a quantitative determination of the value of the B field. This is important because we already have rough arguments supporting the notion of 10^{12} – 10^{13} G magnetic fields at the surface of neutron stars (the value expected from collapse of a main sequence star of radius $R_0 = 10^{11}$ cm and surface magnetic field 100 G by conserving flux BR^2 to a neutron star of radius $R_n \approx 10^6$ cm). We summarise some of these estimates below⁹. (1) The naive theory of pulsar emission suggests that the pulsar energy loss rate is related to the pulsar radius R , magnetic field B , angular velocity Ω , spin down rate $\dot{\Omega}$ and moment of inertia I by

$$dE/dt \approx I\Omega\dot{\Omega} \approx B^2 R^6 \Omega^4 f(R\Omega/c)/c^3$$

where $f(R\Omega/c)$ is a dimensionless number, probably of order 1. From the observed values of dE/dt for the Crab Pulsar NP0532 and of Ω and $\dot{\Omega}$ for other pulsars one finds $B \approx 10^{12}$ – 10^{13} G. (2) Accretion models of binary X-ray sources such as Her X-1 suggest $B \approx 10^{12}$ to 10^{13} G and require $B > 10^{10}$ G in order for the Alfvén radius to be greater than the stellar radius. (3) Finally, for

dynamical stability of the neutron star, one requires $B \ll 10^{17}$ G.

Many factors play a part in making a quantitative derivation of the magnetic field strength from the observation of the X-ray line. For example, if one assumes that the field has a magnetic dipole configuration, the emission must arise¹ from a region with $\Delta B/B < 0.3$ so that the line is not broadened too greatly, in which case one has $\Delta r/r < 0.1$. Emission arises then from a region within 1 km of the neutron star surface. Second, the emitting region must be optically thin, so that the line is not be broadened, shifted and smeared beyond recognition. The detailed radiative transfer in this region is not known, various theoretical models of such effects notwithstanding^{11–13}. Next, a line emitted from the surface of a neutron star must be corrected for redshift¹⁴ which can range from ~ 0 to as much as ~ 0.6 . (This depends on the mass of the star, its equation of state, and the theory of gravity used. For incompressible matter in general relativity, however, $z_{\max} = 2$.) Fourth, if the magnetic field is of order 10^{12} G or greater, the classical electron cyclotron radiation energy will be $\Delta E = \hbar\nu = (e\hbar/mc)B$ for electrons of $v/c \ll 1$. But for $B \approx 10^{12}$ G, $\Delta E \sim 50$ keV, the energy is not negligible compared to the electron rest mass energy. Looked at another way², as the de Broglie wavelength for a 50 keV electron is greater than its cyclotron radius, a relativistic quantum mechanical treatment of the problem is necessary. For electrons with momentum $p_{\parallel}$ parallel to the field, spin state s and principle quantum number n , the energy levels found as eigenvalue solutions to the Dirac equation are given by

$$E = \pm [c^2 p_{\parallel}^2 + m_0^2 c^4 + e\hbar c B(2n + s + 1)]^{1/2}$$

Thus depending on what spin or principle quantum numbers are chosen, the deduced magnetic field will differ from that derived using a non-relativistic (classical or quantum) treatment. For example, if one considers only n states (with $s = -1$, say) the relativistic and non-relativistic treatments can give results differing by a factor of ~ 2 . If one takes all of these effects into account, the deduced field strength can vary from 10^{12} G (for a neutron star of mass $M \approx 0.6M_{\odot}$, lowest energy level or first harmonic at 25 keV) to about 10^{13} G (for a neutron star of $2M_{\odot}$ and taking the first harmonic as the 64 keV line). It is comforting that this range covers the field range deduced from other arguments. A more precise result would require much more detailed information about the neutron star itself, the accretion rate, the topology of the B field, temperature of the radiating electrons, and a host of other parameters.

Observations in the near future from HEAO 1 should be able to establish the reality, energy pulse fraction and relative strength of one or both of the X-ray line features we discussed here. Perhaps detection of linear or circular polarisation in the lines could test the cyclotron origin. The discovery of lines at similar (but not exactly the same) energy might be expected from other X-ray binary systems containing accreting neutron stars.

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Radioastronomical frequency for interstellar NH^+

SOME spectroscopic frequencies may be calculated more accurately than laboratory experiments can measure them. *Ab initio* molecular orbital calculations can be used to compute molecular properties, but none as accurately as Λ -doubling. This effect gives rise to splittings of molecular energy levels of diatomic molecules and transitions between the Λ -doublets are the sources of some of the most important radioastronomical spectral lines. Here we consider the as yet unobserved but very likely instellar species NH^+ .

Very crudely, Λ -doubling may be thought of as an effect of electron slip as the molecule rotates; the electrons lagging somewhat behind the rotating nuclear frame. This results in the energies of degenerate Π molecular energy levels becoming different, with an energy separation which increases as the rotational quantum number increases. Formally the effect may be described as the influence of Σ electronic states on Π states. The interaction, which is zero to a first approximation, is dominated by spin-orbit coupling, giving non-zero matrix elements between Σ and Π states.

The well-known astrophysical source, OH, was the subject of calculations¹ which would now be thought of as relatively crude, but in wave numbers the agreement with experiment is impressive (observed 0.0788 cm^{-1} ; calculated 0.0810 cm^{-1}). This encouraging result was, in a sense, fortuitous because the origin of the splitting in OH is particularly simple, being an example of Van Vleck's approximation of pure precession where the $^2\Pi$ electronic ground state is only seriously influenced by a single $^2\Sigma^+$ state.

The case of CH is more complex as several states of $^2\Sigma^+$ and $^2\Sigma^-$ symmetry have to be considered, but agreement with experiment³⁻⁵ is close enough for the computations to be within a few MHz of the observed astrophysical frequency.

We have now extended this work to the species, NH^+ , where a further complication enters the calculations. In this case we have to consider not only interacting $^2\Sigma^+$ states but also $^4\Sigma^-$ states. Configuration interaction wave functions and potential energy curves were calculated for the $X^2\Pi$, $a^4\Sigma^-$, $A^2\Sigma^-$ and $C^2\Sigma^+$ states of NH^+ using the Alchemy system of computer programs⁶. Using these wave functions and potential energy curves the interaction between the $X^2\Pi$ state and the $a^4\Sigma^-$ state was treated numerically, whilst the interaction with the $^2\Sigma^+$ states was calculated using second-order perturbation theory. It is important that because of the influence of the $^4\Sigma^-$ state, the Λ -doubling in the $X^2\Pi$ state of NH^+ cannot be interpreted in terms of the conventional constants p and q and each rotational level must be treated separately.

The results of these calculations predict Λ -doubling in the lowest rotational level of $^{14}\text{NH}^+$ to be 13.625 MHz. This may be compared with the best laboratory experimental value of $13,520 \pm 300\text{ MHz}$ for which special measurements were made⁷.

The splittings to be expected in $^{14}\text{ND}^+$ and $^{15}\text{NH}^+$ together with values in excited rotational levels for which accurate experimental measurements are not available will be published elsewhere with fuller details of this work.

Our predicted frequency of NH^+ is within the range of available radiotelescopes and the calculations could provide the first example of a case where theory has predicted a radiosource rather than merely confirmed its identification.

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Irreversibilities in the mechanism of photoelectrolysis

PHOTODECOMPOSITION of water using semiconducting electrodes is being widely investigated. Using a combination of theoretical methods and experimental results we have analysed the sequential processes involved in this phenomenon and show here that several steps are irreversible. Some of the consequences of these irreversibilities are discussed. A theory of electronic charge transfer between electrodes and aqueous electrolytes, based on an equilibrium approximation by Marcus¹, seems to be valid for metal electrodes. Gerischer² has considered both metal and semiconductor electrodes and concluded that in general the electron is isoenergetically injected (tunnels) from the occupied band state of the electrode into an unoccupied state of the electrolyte. Rearrangement of the molecular environment then occurs, irreversibly changing the energy of the electronic state of the electrolyte. Finally, reverse tunnelling from the relaxed state back into the electrode may occur.

The two states of occupancy in the electrolyte constitute a redox couple. For metals the forward and reverse currents become equal as equilibration occurs; thus, the Fermi level of the metal adjusts to the redox potential of the redox couple of the electrolyte. For semiconductors, electronic charge transport involving different bands (electrons in the conduction band and positive holes in the valence band) have been separately considered². This situation can lead to either an equilibrium or a non-equilibrium charge transfer process between semiconductors and electrolyte. The equilibrium approximation frequently appears in analyses of electronic charge transport between semiconductors and electrolytes. In particular, Gerischer³ has invoked the quasi-Fermi level concept to describe the charge carrier concentrations at the semiconductor-electrolyte interface under illumination.

Here we point out some irreversible aspects of charge transfer across semiconductor-electrolyte interfaces for photogenerated minority carriers, and emphasise the importance of these irreversibilities in explaining photoelectrolysis⁴⁻⁷ and the mechanism of the operation of the photochemical diode⁸. Furthermore, we believe that as a consequence of these irreversibilities, the quasi-Fermi level concept and the associated thermodynamic arguments are of limited validity for high efficiency photoelectrolysis. Irreversibility, has previously been proposed to explain reduction of ferricyanide at a ZnO semiconductor electrode⁹.

The energy level diagram for the *n*-type semiconductor electrode-aqueous electrolyte interface involved in photoelectrolysis is shown in Fig. 1. The essential features of the semi-conductor-electrolyte junction are from Nozik¹⁰; the electronic states of water are from Williams *et al.*¹¹. The bending of the conduction and valence band edges, E_c and E_v respectively, are determined by the space charge arising

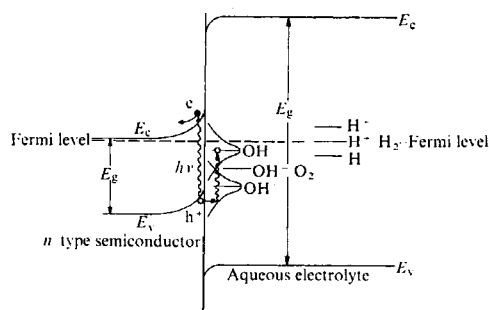


Fig. 1 Energy level diagram of n -type semiconductor (TiO_2 , $E_g = 3\text{eV}$) in contact with aqueous electrolyte ($E_g \approx 9\text{eV}$) showing energy bands of water and its extrinsic states. The energy levels of the extrinsic states have a distribution due to thermal fluctuations in the solvation structure; this is shown for the OH^0 and OH^- states.

from the difference in work functions of the semiconductor and of the electrolyte. The extrinsic states, H^+ and OH^- , are approximate, having been estimated from continuum theory¹¹. At the interface the macroscopic dielectric constant and the continuum approximation for small ions in solution are not strictly valid. The rearrangement energies can be similarly estimated, but for our purposes are taken from Henglein¹².

The sequence of events at the n -type semiconductor anode during photoelectrolysis are: (1) the incident quantum of radiation, $h\nu$, is absorbed in the semiconductor and thus creates a conduction electron and a valence band hole; (2) the electron, which is the majority carrier with energy very near the Fermi level, is displaced by the band bending into the external circuit, and the positive hole which is the minority carrier, and as such has energy available for radiative transitions or chemical changes, is displaced by the band bending towards the interface with the aqueous electrolyte; (3) the positive hole tunnels from the valence band edge into OH^- molecular ions at or near the interface; (4) a neutral OH^0 species is formed which causes the orientated H_2O molecules which surround the OH^- ion to relax, perturbing this extrinsic electronic state closer to the Fermi level; and finally (5) the chemistry to form O_2 proceeds according to the overall reaction: $4\text{OH}^0 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$.

Experimental data on the photoelectrolysis of water using $n\text{-TiO}_2$ anodes and Pt cathodes indicate that the quantum efficiency (that is, the fraction of absorbed photons producing charge carriers which are injected into the electrolyte to propagate the anode reaction) is high, especially if the band bending in the space charge region is reasonably large and the depletion width (w) is greater than the absorption depth ($1/\alpha$), where α is the absorption coefficient in cm^{-1} . Thus, for example, at $3,100\text{ \AA}$, where $\alpha = 6 \times 10^5\text{ cm}^{-1}$ for TiO_2 , the quantum efficiency for the oxidation of water to O_2 at the TiO_2 anode is reported to be 1.0 (A.J.N. and R. R. Chance, unpublished and ref. 7). Under these conditions, the band bending is 0.9 eV (ref. 7), the absorption depth is $170\text{ \AA}$, and the depletion width is calculated to be $200\text{ \AA}$; the latter calculation is based on a carrier density of $2 \times 10^{19}\text{ cm}^{-3}$, which is typical for reduced TiO_2 crystals. At longer wavelengths, where the absorption depth increases, the quantum efficiency decreases. Thus, at $3,484\text{ \AA}$, where $\alpha = 1.1 \times 10^5\text{ cm}^{-1}$ and the absorption depth is $910\text{ \AA}$, the quantum efficiency was measured to be 0.7 with a band bending of 0.9 eV . At this wavelength, the quantum efficiency decreases to values of 0.6, 0.5, and 0.2 at band bending values of 0.7 eV , 0.5 eV , and 0.3 eV , respectively.

The kinetics of photoelectronic injection from a semiconductor into an aqueous electrolyte depend on five time constants: τ_{recomb} , the time constant characterising the recombination rate of photogenerated electrons and holes; τ_t , the tunnelling time for charge transfer from the band

states of the semiconductor to the localised extrinsic states of the aqueous electrolyte; τ_r , the relaxation or rearrangement time characteristic of the extrinsic states of the aqueous electrolyte in going from the equilibrium condition of polarisation with the state unoccupied to the equilibrium condition with state occupied; τ'_t , the tunnelling time for charge transport from the relaxed occupied electrolyte state back into the semiconductor bands; and τ_c , the time constant for the occupied electrolyte state to undergo annihilation by chemical changes. Depending on the relative magnitudes of these time constants, various steps in the charge transfer may approach either equilibrium or irreversible conditions.

Under conditions of high quantum efficiency (0.8–1.0) as discussed above, the time constants are constrained as follows: $\tau_{\text{recomb}} > \tau_t$, $\tau'_t > \tau_t$, and $\tau'_t > \tau_c$. In other words, each photogenerated minority carrier must survive each step without appreciable probability of recombination with majority carriers, nor return to the semiconductor after injection. The latter condition is consistent with the low exchange currents observed with semiconductor electrodes¹³ and is understandable from the relaxation resulting in the occupied electrolyte state lying within the band gap of the semiconductor (see Fig. 1). The former condition is consistent with estimated values of τ_t and τ_{recomb} . The maximum tunnelling time, τ_t , can be estimated using the standard model of tunnelling¹⁴ from a potential well having a width of $200\text{ \AA}$ (depletion width), a barrier height of 3 eV (difference between valence band edge of the semiconductor and that of intrinsic water), and a barrier thickness of $10\text{ \AA}$ (distance of extrinsic OH^- levels from semiconductor surface). This thickness is greater than that of adsorbed OH^- ions, but less than the average inter-anion distance in the bulk electrolyte; the tunnelling rate is insensitive to the barrier thickness up to about $30\text{ \AA}$. In this model, carriers which have not undergone full intraband relaxation in the depletion layer ('hot' carriers) may also be injected into the electrolyte. The importance of band bending here is to confine the carriers within w , which increases the tunnelling rate. This calculation yields a value of about $5 \times 10^{-13}\text{ s}$ for τ_t . This is much faster than the estimated value of 10^{-10} s for τ_{recomb} which is calculated for bulk recombination in a direct band gap semiconductor with a majority carrier density of $2 \times 10^{19}\text{ cm}^{-3}$ (ref. 15). Surface recombination rates are more difficult to estimate.

Even faster tunnelling times would be obtained if the OH^- ion is taken to be closer to the semiconductor surface than $10\text{ \AA}$. For such strongly adsorbed molecular ions, a model involving a combined semiconductor-molecular ion eigenstate may be considered; this permits direct photoinjection of the hole to the OH^- ion without the necessity of a tunnelling process.

The concept of quasi-Fermi levels has been widely used in solid state physics in describing photoconductive, photovoltaic and semiconductor devices. The quasi-Fermi levels are different for electrons and for holes and describe the separate occupational probabilities for these two types of electronic particles among their respective band and defect states. The concept depends on local statistical detailed balance between the rates of creation and of annihilation of electrons and holes. For photoelectrolysis with high quantum efficiency, as noted above, local statistical detailed balance of electrons and holes is not achieved in the semiconductor near the electrolyte interface because the minority carriers are so rapidly depleted by tunnelling into the electrolyte; that is $\tau_{\text{recomb}} > \tau_t$. Therefore, the charge transfer process is better approximated as individual events. With the reverse tunnelling (exchange current) negligible, that is, $\tau'_t > \tau_t$, the charge transfer corresponds to irreversible photoinjection. This is similar, as regards irreversibility, to photoelectric emission into a vacuum; but the origin of the irreversibility is, of course, quite different.

In Fig. 1, the Fermi level of the semiconductor is shown above that of the OH^-/O_2 redox level, and it is equal to the Fermi level of the electrolyte. As O_2 is evolved at the semiconductor anode under illumination, and the concentration of OH^- and other intermediate species accumulate, the reaction at the semiconductor-electrolyte interface will tend to equilibrate with the OH^-/O_2 redox level. This equilibrium will occur if the chemical depletion of OH^- is slow compared to the relaxation time of OH^- to its equilibrium state in the electrolyte, that is, if $\tau_c > \tau_r$. With finite activation energy this condition is true, so that the anodic reaction in the electrolyte proceeds in approximate equilibrium with the OH^-/O_2 redox level; the absolute value of τ_r is 10^{-11} (ref. 16).

A new and simple device for the photochemical conversion of optical energy has recently been announced⁸. These devices have been labelled 'photochemical diodes', and consist of monolithic structures comprising either a semiconductor and a metal region or two different semiconductor regions (one *n*-type, the other *p*-type) which are connected through ohmic contacts. The latter is called a *p-n* heterotype photochemical diode, and it can exhibit voltage enhancement when the electron affinity of the *n*-type semiconductor is greater than the electron affinity of the *p*-type semiconductor⁸.

In accordance with the above discussion, the *p-n* photochemical diode can be described as a photocathode and photoanode, back-to-back, respectively irreversibly photo-injecting electrons and positive holes as minority carriers into extrinsic states of the electrolyte and with the majority carriers recombining in the ohmic contacts.

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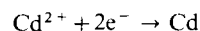
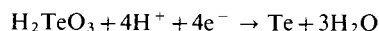
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The films of CdTe were prepared by the simultaneous electrodeposition of Cd and Te on a Ti base. Presumably the process is similar to that for CdSe film formation and involves the following reactions at the cathode:



The CdTe films were prepared galvanostatically in a two compartment cell in which 2 M H_2SO_4 was used as the anolyte, a solution of 0.01 M H_2TeO_3 , 1.5 M H_2SO_4 and 0.27 M CdSO_4 as the catholyte, platinum as the anode and titanium as the cathode. The compartments were connected by a KCl-agar salt bridge.

Before use the titanium was prepared by lightly sanding the surface to remove any oxide layer and then washed in hexane to degrease the surface. The best films were prepared from a stirred solution with the composition given above and using a current density in the range 30-40 mA cm^{-2} . The films formed were black and seemed uniform in texture and thickness, an upper limit for which was 8 μm . The films adhered well to the titanium. Heat treatment at 250 °C for 16 h increased the photovoltage obtained in the photoelectrochemical cell and also reduced its series resistance.

The films were tested in a nitrogen-purged electrolyte containing 1 M NaOH, 1 M Na_2S and 1 M S. The open-circuit voltage was 0.34 V, the short-circuit current 2.6 mA cm^{-2} and the fill factor 25%. The best power conversion efficiency obtained so far is 0.4% under an irradiance of 50 mW cm^{-2} of white light from a xenon lamp with a Schott KG4 filter.

When argon-purged 5 M NaOH, containing 0.03 M partly oxidised sodium telluride, was used instead of the sulphide-sulphur solution, the photovoltage at open-current was 0.29 V, the short-circuit current density, 2.2 mA cm^{-2} and the fill factor 27%. The power conversion efficiency for the same light as before was 0.4% when the irradiance was taken as that incident on the solution. Because the solution absorbed light, the power conversion efficiency with regard to light incident on the CdTe film was between 2 and 4%. Efficiencies can no doubt be improved by varying the deposition conditions and by using cells in which there is less absorption by the solution.

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Photoelectrochemical cell with cadmium telluride film

ELLIS *et al.*¹ reported studies of single crystal CdX based photoelectrochemical cells when X = S, Se or Te and found that the best efficiencies were obtained when CdTe was used as the photoelectrode. Photoelectrochemical cells using thin films of CdS and CdSe have been reported by Miller and Heller² and by Hodes *et al.*³ In this paper, we report on a photoelectrochemical cell using a thin polycrystalline film of CdTe.

'Low scatter' conduction of electric charges through lamellar solids

MATHEMATICAL theories about the conduction of electricity through solids have been detailed for conventional metals¹. It is generally necessary to combine assumptions about the concentration of charge carriers with calculations about their probabilities of being scattered during flow. For synthetic metals the predominant scattering processes may be quite different, however. This may be so in lamellar solids after they have been converted into good electrical conductors by intercalation of electron donor or electron acceptor molecules between the layers. Such molecules are thought to form charge

transfer bonds with the two-dimensional macromolecules that constitute the layers in the parent solids before intercalation. Even static aspects of probable charge transfer bond formation have not always received complete quantum mechanical formulation. Dynamic aspects (which may influence scattering processes during current flow) are even less completely described. This letter points out that scatter probabilities of current carriers injected by charge transfer may be markedly lower than in the more familiar 'natural' three-dimensional metals. This may help to explain high electrical conductivities of lamellar synthetic metals.

Following an original hypothesis for lamellar compounds between graphite and a typical electron donor partner such as potassium, or a typical electron acceptor partner such as molecular bromine³, the remarkable increases of electrical conductivity, observed on forming intercalation compounds starting with crystalline layer lattices, were attributed to consequences of charge transfer bond formation between the giant planar carbon networks and electron donor or electron acceptor partners. Such resonance bonding may be comparatively weak, but until recently the fractional charge transfer f between partners has been only indirectly estimated. In alkali metal compounds with graphite³, just as with smaller aromatic molecules^{4,5}, volume occupancy calculations suggested that fractional charge f transfer must be near unity, but with electron acceptor partners a considerably smaller transfer seemed likely. These estimates were, however, confused by similarities in behaviour of synthetic metals⁶ such as dilute graphite-bromine, formed by direct intercalation of the halogen molecules, when compared with lamellar compounds such as graphite bisulphate formed by electrochemical oxidation⁷. In these electrochemical compounds the total quantity of electricity abstracted per g atom of graphite oxidised is accurately known. Plausibly this gives a direct measure of charge transfer fraction for electrochemical compounds. If valid, such an argument would be helpful by analogy, as electrochemical compounds are known in considerable variety⁸.

Recent optical studies⁹ give direct evidence, however, that in a lamellar compound, such as graphite-bromine, fractional charge transfer does not exceed a few tenths of 1% as most of the molecules of Br₂ are 'un-ionised'. This finding has vital consequences when interpreting the large increases of electrical conductivity (in the direction of the layer planes) on forming these synthetic metals. The structure and electrical properties of bromine-graphite are well known. A steep increase of conductivity is observed on intercalation of only very small mole fractions of Br₂ (ref. 6). The character of such increases is general for many kinds of intercalates, which at first (in dilute synthetic metals) occupy only a small fraction of the layers in the lamellar solid. Conductivity increases should apparently not be attributed specifically to any very large increase in the number of charge carriers made available per atom of carbon in the giant network conductors. To account for the high electrical conductivity of lamellar synthetic metals, the essential assumption seems to be¹⁰ that the comparatively few additional charge carriers transferred experience quite exceptionally 'low scatter' in the directions of the layer planes. At the present stage of research it might be misleading to term these lamellar solids 'super'-conductors. Cooperative effects between charge carriers are not excluded, but 'low scatter' describes the effects observed sufficiently.

The preceding remarks have been prompted by the considerable body of information on layer intercalates in graphite¹¹; however, 'low scatter' mechanisms of electrical conduction may be identifiable in other two-dimensional conductors, such as other lamellar intercalates. Even for one-dimensional, 'tunnel' or fast ion conductors scattering mechanisms seem likely to be much less effective than in conventional metals which are at least approximately three-dimensional. Whatever the nature of the solid framework in which localised charge transfer takes place, it may be important that the mobility of the molecular units from which transfer occurs remains high. They can then

participate in processes of momentum and energy exchange, together with the lighter charge carriers, when an applied voltage gradient leads to a drift of electric charge.

'Low scatter electrical conduction' parallel to the network has already been suggested by several highly unusual voltaic effects in lamellar compounds of graphite⁶. In particular, the Remote Voltaic Effect could (in one interpretation) be attributed to beams of charge carriers travelling in the lamellar solid but with each beam largely confined between its neighbouring pair of giant carbon networks; a physical analogy would be with a beam of light travelling down a curved film of glass, and unable to escape because of its total internal reflection at the air-glass surface. Unlike the transparent glass, in the lamellar compounds of graphite some general scattering seems unavoidable. Remote voltaic effects have, nevertheless, been detected over distances of several centimetres. By rigorous selection of the parent graphite specimens to be as near perfect as possible before intercalation, all these novel voltaic effects are generally more prominent and thus easier to observe. In addition to using selection criteria previously described, it is desirable to choose parent materials for which the magneto-resistance coefficient is high¹², and for which the intercalation threshold is very low¹³. Though methods for doing this are less well known, the importance of achieving low scatter by minimising structural imperfections probably also applies to diverse synthetic metals other than those based on graphite.

Study of all these effects has been greatly eased by the construction of a novel pulse meter¹⁴. This readily permits voltage gradient measurements using very large current densities up to 40,000 A cm⁻². Remote voltaic effects and other voltaic anomalies in lamellar conductors can be more readily followed with the much larger pulse currents that can now be used. The search for critical or threshold parameters is now also facilitated. Anomalous effects are not observed in the parent graphite despite its pronounced lamellar structure, but when the layers become separated by intercalation using diverse partner molecules, the marked increase in electrical conductivity and the appearance of unusual voltaic effects go side by side.

Even mass movement of the molecules themselves, acting as charge transfer partners, occurs with remarkable ease within the confinement of any intercalated layer. Some evidence^{10,11} indicates that the 'ripple potential' affecting such predominantly two-dimensional movement of molecules in a layer lattice seems far less restrictive than in most cases of mass diffusion in three-dimensional lattices and even compared with the extreme case of solid ionic good conductors (the so-called 'solid electrolytes').

Confinement to two-dimensional layers or even greater 'tunnel' restrictions on movement seems to affect markedly the electric charge carriers. Present data suggest that the unusual voltaic effects as well as the high electrical conductivity of lamellar synthetic metals are mainly due to 'low scatter conduction' of these charge carriers in the directions of the layer planes in graphite intercalates.

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X-ray photoelectron spectroscopic evidence for trapped oxygen species in irradiated NaClO_3

X-RAY Photoelectron Spectroscopy (XPS) has many advantages for studying, *in situ*, the photo-induced decomposition of inorganic molecular ions; this is shown by our data on the radiolysis of NaClO_3 , sodium chlorate, using the standard $\text{Mg (K}\alpha)$ anode [$1,254 \text{ eV}$, $\lambda \approx 10\text{\AA}$] in our AEI ES200B photoelectron spectrometer. We have already examined¹ the decomposition kinetics of NaClO_4 and NaClO_3 , when the final products of irradiation were found to be a defect NaCl phase together with molecular oxygen (detected by mass spectrometry). We report here some further low temperature XPS studies on the NaClO_3 system, where strong evidence for the presence of trapped oxygen species in the initial stages of irradiation has emerged.

Powdered samples were examined in the temperature range 83–293 K using a liquid-nitrogen cooled sample-probe coupled with a resistance heater and null-point thermistor giving automatic control within $\pm 5 \text{ K}$. A base pressure of 4×10^{-10} torr in the sample chamber was achieved by previous baking of the vacuum system. An MAT AMP3 quadrupole mass analyser was connected directly to the sample chamber, and pressures were measured with a cold cathode Penning gauge situated $\sim 10 \text{ cm}$ from the sample. At any given temperature the photoelectron spectrum of an NaClO_3 sample was recorded at various times of exposure to the exciting X radiation. The core-electron levels from chlorine [$\text{Cl}(2p)$] and oxygen [$\text{O}(1s)$] were of most interest although we also routinely recorded the $\text{Na}(2s)$, $\text{C}(1s)$, $\text{Cl}(2s)$ and some sodium Auger peaks as calibration checks.

The rate of decay of the original $\text{Cl}(2p)$ and $\text{O}(1s)$ photoelectron peaks followed 1st-order behaviour, confirming our earlier results at 253 K¹. At temperatures in the range 113–233 K the $\text{O}(1s)$ region developed an additional peak at $\sim 5 \text{ eV}$ lower kinetic energy (higher binding energy), which grew to a limiting value with X-ray dose before decaying slowly (see Fig. 1a and 1b). The original $\text{O}(1s)$ peak was also observed to broaden with X-ray dose as its intensity declined. A sample of irradiated NaClO_3 with an appreciable low kinetic energy $\text{O}(1s)$ component was allowed

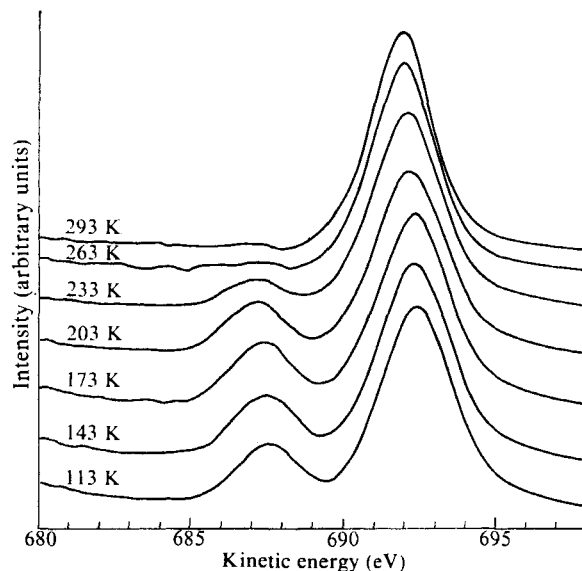


Fig. 2 Effect of raising the temperature on the $\text{O}(1s)$ photoelectron spectrum of a sample of irradiated NaClO_3 , originally at 113 K. Sample chamber pressures were used to ensure equilibrium at each temperature.

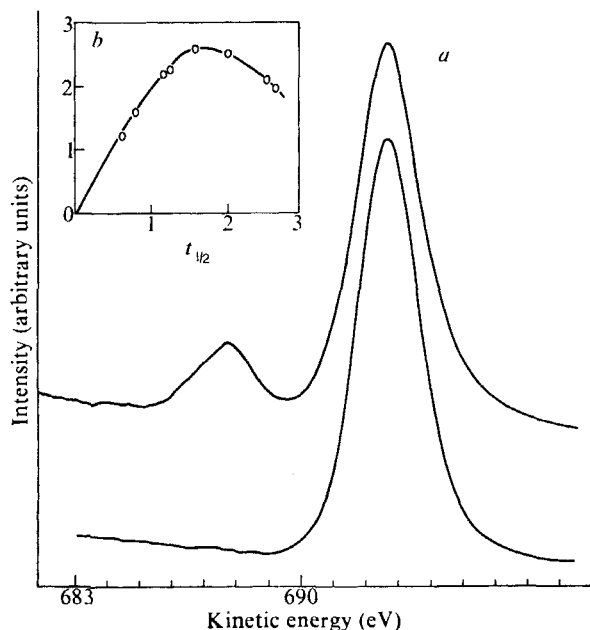
to warm gradually to 293 K (see Fig. 2), when this feature was seen to decrease slowly, with a concomitant increase in the sample chamber pressure, due to $\text{O}_2(\text{g})$, (confirmed by intense peaks at $m/e = 32$ and 16 in the mass spectrum of the sample chamber atmosphere).

For irradiation periods much larger than the half-life of ClO_3^- , (when the $\text{Cl}(2p)$ signal associated with the molecular ion had virtually disappeared), the main $\text{O}(1s)$ signal remained roughly constant at $\sim 20\%$ of its initial value. A consistent interpretation of these results can only be obtained if the smaller $\text{O}(1s)$ component is assigned to an oxygen species incorporated by the crystal lattice. The possibility that it is due to a weakly-bound oxygen molecule adsorbed on the surface was ruled out by studying the $\text{O}(1s)$ regions of other ionic crystals which are chemically stable to ionising radiation (NaCl , Na_2SO_4). The only peaks observed had binding energies similar to the main $\text{O}(1s)$ component in NaClO_3 ; thus the smaller $\text{O}(1s)$ component arises solely from the induced decomposition of the molecular ion.

An electron spin resonance (ESR) study² on irradiated KClO_3 postulated the formation of molecular anions O_2^- ; which can be accommodated at vacant anion sites, providing an electron trap equivalent to the F-centre in NaCl . They exist in appreciable concentrations and show a fair degree of thermal stability. The low-intensity $\text{O}(1s)$ component cannot be assigned to this species, however, since its intensity decreases as the decomposition of NaClO_3 proceeds, and is also very sensitive to the sample temperature. We have, therefore, assigned the residual component of the main $\text{O}(1s)$ peak to O_2^- with a binding energy similar to that reported for oxides and chemisorbed oxygen species³.

The second low-intensity component at higher binding energy implies a small negative charge per atom. The average volume of the interstitial site in NaClO_3 (ref. 4) is $\sim 15\text{\AA}^3$ which is large enough to accommodate an oxygen atom of $\sim 12\text{\AA}^3$ volume, (calculated from the van der Waals radius). Similar considerations for the more closely-packed NaCl ⁵ structure yield an interstitial cavity of only $\sim 8\text{\AA}^3$. We cannot assign the low intensity component simply to an interstitial oxygen atom, however, because the multiplet splitting of the $\text{O}(1s)$ level corresponding to the $^2P, ^4P$ states of the ionised atom can be calculated⁶ to be greater than 2 eV. Our observations indicate a multiplet splitting of less than 0.8 eV, which implies considerable delocalisation of unpaired spin density away from this centre. We propose that this is achieved by the formation of a loosely-bound $\text{O}_2 \dots \text{O}$ species, between an interstitial O and an O_2^- ion at an

Fig. 1 a, Lower trace: initial $\text{O}(1s)$ photoelectron spectrum of NaClO_3 at 203 K; upper trace: same region after $\sim 180 \text{ m}$ irradiation. b, Intensity of low kinetic energy $\text{O}(1s)$ peak as a function of $t_{1/2}$ (obtained from $\text{Cl}(2p)$ intensity data).



adjacent normal lattice site. The formal similarity of this species to the well-known ozonide anion O_3^- , the existence of which in irradiated $NaClO_3$ has been proposed from ESR studies^{7,8} supports our assignment. It should be stressed, that the formation of the $O_2 \cdots O$ complex is strongly dependent on the volume of the interstitial cavity, which must only just be large enough to trap the O atom; thus we have failed to detect any O-trapping in the low temperature radiation-induced decomposition of $NaNO_3$ (R. G. C. and J. L. unpublished).

If we suppose a step-wise process for the decomposition¹ involving $ClO_3^- \rightarrow ClO_2^- + O$ as the initial step, then the majority of the oxygen atoms will diffuse through the lattice (low trapping probability for interstitial O) to form molecular oxygen, which either escapes from the lattice, or is trapped at anion sites as O_2^- . On continued irradiation interstitial oxygen atoms will also combine with O_2^- , resulting in a slow rise in the high binding energy $O(1s)$ peak. At long irradiation times, however, the number of oxygen atoms produced will decrease, which, when combined with a structural change to the $NaCl$ phase (with a smaller interstitial cavity), will lead to the observed decrease in intensity. The warming-up experiment, shown in Fig. 2, can thus be explained in terms of the decomposition of $O_2 \cdots O$, and thermal diffusion of oxygen atoms which combine to form molecular oxygen.

We also observed that irradiation at the lowest temperature attainable (~ 85 K) led to no O trapping; also samples irradiated at higher temperatures and containing significant amounts of trapped O behaved exactly as in the warming up experiments on cooling to 85 K, that is, disappearance of the high binding energy $O(1s)$ peak accompanied by the evolution of O_2 . This suggests that a structural change occurs, to a more closely-packed lattice, in the region of 85 K.

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Carbon fibre structure by electrolytic etching

STUDIES of polyacrylonitrile (PAN)-based carbon fibres have demonstrated a concentric three-zone skin-sheath-core structure¹⁻⁷ originating in the oxidation of the precursor fibre before pyrolysis heat-treatment^{5,6}. I describe here an electrolytic oxidation treatment that is effective in showing up different zones in carbon fibre sections.

PAN fibres, 1.5 denier were oxidised in air at 230 °C for either 30 min or 2 h, and heat-treated at 1,000 °C, 1,400 °C or 2,500 °C giving in total six fibre types. Single tows of each fibre type were mounted in epoxy resin blocks and 3-mm thick disks were cut normal to the fibre axis. The disks were polished on one side and sputter-coated with gold on the other to make electrical connection to all fibre ends. For electrolysis the polished face was made anode in a 2 M sulphuric acid bath with carbon cathode. Longitudinal fibre sections were also prepared in a similar manner. In these, conduction through the disk is by fibre-to-fibre contact, and it was necessary to use a high-volume fraction composite of commercial type 2 PAN-based fibre rather than single tows in order to achieve sufficient conductivity. The electrolytic treatment is basically anodic oxidation. Average

current density at the fibre ends was limited to 140 A m⁻² for convenient treatment times, though neither acid concentration nor current density were found to be critical. Specimens were removed after intervals of 10–60 s, and rinsed in distilled water before microscopy.

In the initial stages of the treatment, fibre surfaces developed colours which later dulled as oxidative removal of carbon formed relief. The colours are believed to arise from interference in a thin surface layer with different optical properties, formed by intercalation of sulphuric acid into the fibre^{7,8}. As the optical path difference inferred from the interference colour increases linearly at a rate of 0.18 nm per C/m², the advancement of any particular area in the interference series is an indicator of its extent of reaction in forming an intercalation compound. The colours extend only to second order blue, which is maintained after relief is developed. Specimens were examined soon after etching as the colours fade over a period of several weeks at room temperature.

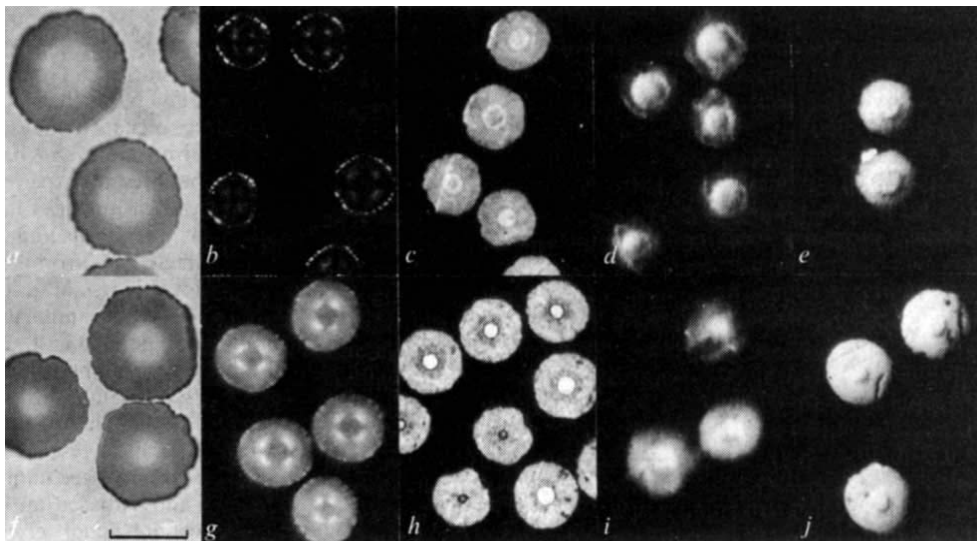
For all sections cut normal to the fibre axis the sheath etched more rapidly than the core, and increasingly so for higher heat-treatment temperatures (HTT) (Fig. 1*c–e, h, j*). In every case etching in the sheath decreases outwards, but there are differences in this gradation between HTT's. A dark ring of very etched material, approximately 1 µm wide, immediately surrounds the core in 2,500 °C HTT fibres, while the remainder of the sheath is uniformly etched at a lower rate. Fibres heat-treated at 1,000 °C and 1,400 °C show a more even outward decrease in etching of the sheath which is shown clearly by the (coloured) concentric rings in Fig. 2*b*, and the smooth contours in Fig. 1*d, e, i, j*. The core etched uniformly for all heat treatments and is seen as a plateau in fibres showing relief. In longitudinal sections both sheath and core etched uniformly, but the core more rapidly than the sheath, a reversal of the order in transverse sections (Fig. 2*a*).

Watt and Johnson⁵ have observed oxidation zones in thin sections of the PAN fibres from which the carbon fibres examined here were made. The thickness of the darker oxidised zone corresponding to the sheath increases with the square root of oxidation time (Fig. 1*a, f*) and after heat treatment the structure is apparent in polarised light^{2,3,6} (Fig. 1*b, g*). Direct comparison of the zone dimensions in oxidised and carbonised fibres is not possible because of the shrinkage from 13 to 8 µm diameter, but the relative sheath thicknesses correspond. In polarised light a Maltese cross is seen in both sheath and skin, whereas the core is optically isotropic. The contrast is lower in 1,000 °C and 1,400 °C HTT fibres but the pattern remains the same. These patterns have been interpreted in terms of the *c*-plane orientation about the fibre axis^{2,3}, which for the 8 µm PAN-based fibres is thought to be concentric in the skin, radial in the sheath and random in the core. This model agrees well with the structure seen on fracture surfaces² and in plasma-oxidised fibre sections¹, where radial striations may be seen in the sheath, and circumferential rings in the skin. Orientation is less apparent in these surfaces below 2,000 °C HTT, and it is noted that only in Fig. 1*h* (2,500 °C HTT) are there dark radial streaks in the sheath.

It is becoming evident that the sheath/core distinction is not simply one of *c*-plane orientation. Electron microscopy of crushed fibre fragments⁴, and electron diffraction studies of laper-thinned fibres² show decreasing layer-plane misorientation and decreasing crystallite stacking height from the fibre centre outwards. A uniform outward decrease is associated with the lower heat-treatment temperatures, but for 2,500 °C HTT fibre the transition takes place in a narrow 1-µm wide band; patterns which are mirrored in the etched fibre surfaces (Fig. 1*c, e, h–j*).

The thickness of the intercalated layer might be expected to vary with the inclination of the *c* planes to the surface, rising to a maximum when they are normal. If there are preferred circumferential and radial crystallite orientations any non-diametric longitudinal section will show some variation of the mean *c* plane to surface angle within zones, which might be reflected in variation of the etching rate across the zones. In fact, the zones etch uniformly (Fig. 2*a*), showing that the technique is insensitive to such orientation as may exist. Differences in crystallite mis-orientation in sheath and core will give rise to differences in plane

Fig. 1 Transverse fibre sections, air-oxidised at 230 °C for 30 min (*a-e*) or 2 h (*f-j*). *a,f*, Thin sections of PAN fibre before heat-treatment. *b,g*, Polarised light, 2,500 °C HTT. *c,h*, Etch colours in 2,500 °C HTT fibres. *d,i*, Etch relief in 1,400 °C HTT fibres. *e,j*, Etch relief in 1,000 °C HTT fibres. Scale bar, 10 μm .



edge density in transverse sections, but these do not seem able to account for the contrast in etch rates.

In longitudinal sections intercalation will initially take place in crystallites presenting *c*-plane edges to the surface, and the depth of penetration will be limited by the crystallite width. A higher etching rate will then be associated with wider crystallites. The core is known to have greater crystallite thickness than the sheath, and it is possible that the effective crystallite width is also greater, which would account for its higher rate of etching.

Because of the high axial *c*-plane alignment all crystallites in transverse sections will be suitably oriented for intercalation. But, the rate of intercalation is expected to be greater in the less misoriented sheath where there is greater separation of lattice distortions hindering penetration. It is probable also that axial curvature and folding of layer planes produces channels in which intercalation is facilitated, and a higher density of these may accompany the smaller sheath crystallite thickness.

These ideas come some way to explaining the reversal of sheath and core etch rates in longitudinal and transverse sections. Although the etch patterns can only be correlated with what is already known of fibre fine structure, the technique is worth pursuing as a simple and sensitive monitor of morphological variations arising during the processing of PAN fibre to carbon fibre. As such it is an aid in relating strength properties to structure, and for this application a particular advantage of

current controlled etching is the fact that insulating resin matrices are not attacked.

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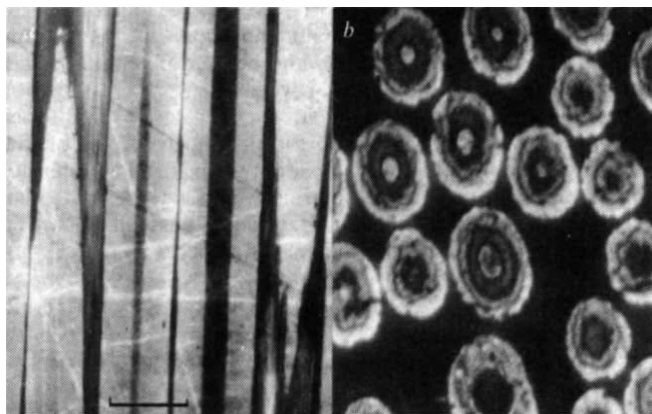
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Evidence for reptation in an entangled polymer melt

ENTANGLEMENTS, their nature and their role in the dynamic properties of concentrated polymer solutions and melts are not well understood^{1,2}. The classical molecular view of entanglements has been one of rope-like intermolecular couplings at a number of points along the length of a molecule; molecules in motion would drag past these couplings, the essential effect being one of enhanced friction^{1,3}. There has been a growing realisation that this model is inadequate^{2,4,5}. The essence of the problem, rather, seems to be that of the topological restrictions imposed on the motion of each polymer molecule by its neighbours: movement of a given polymer chain is constrained at the points of entanglement or intersection with adjacent chains³. Theoretical treatment of the topological problem is difficult⁶, and has met only with limited success⁵. An interesting proposal regarding the motion of molecules within entangled polymer systems has been put forward by De Gennes^{4,7}: according to this, the motion of a given polymer molecule is confined within a virtual 'tube' defined by the locus of its intersections (or points of 'entanglement') with adjacent molecules (Fig. 1). The molecule is constrained to wriggle, snake-like, along its own length, by curvilinear propagation of length defects such as kinks or 'twists'⁸ along the tube; this mode of motion has been termed reptation⁴ (from reptile). Reptative motion clearly satisfies the central requirement of entangled systems: that of the non-crossability by a given chain of the contours of its adjacent

Fig. 2 Etched sections of commercial type 2 PAN-based carbon fibres. *a*, The two centre fibres show a dark core more etched than the sheath. *b*, Concentric (coloured) rings in the sheath indicate a decrease of etching outwards. Scale bar, 10 μm .



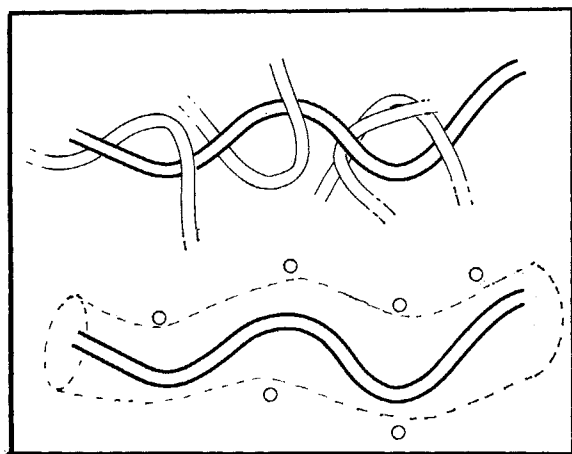


Fig. 1 A polymer chain in an entangled melt (or concentrated solution) may be regarded as being within a virtual 'tube' defined by the locus of its intersections with other melt molecules, as indicated. The obstacles defining the 'tube' in the lower figure are sections through the melt molecules in a plane parallel to the paper. For clarity, the density of segments shown is much lower than would be the case in a real melt.

neighbours. In a real polymer melt the topological environment of any given molecule (that is, the virtual 'tube' surrounding it) will itself change with time. This is because the adjacent molecules defining it are themselves mobile. If this reorganisation is sufficiently slow then the translational motion of the enclosed molecule will be effectively curvilinear (reptative). Consideration of the problem⁹ suggests that this will be the case in an entangled system. One then expects translational diffusion to be dominated by reptation. There is no direct experimental evidence supporting the physical reality of curvilinear motion in entangled polymer systems. I report here the results of experiments on diffusion within a polyethylene melt critically designed to test the reptation concept.

One outcome of constraining a polymer chain of random walk configuration to diffuse, by Brownian motion, within a confining (virtual) tube, is that the translational diffusion coefficient D scales as

$$D \propto M^{-2} \quad (1)$$

where M is the molecular weight. This may be readily proved⁴, and is a direct consequence of imposing a random walk configuration on the essentially one-dimensional curvilinear diffusion of the molecule along its own contour.

Previous studies of self-diffusion in concentrated polymer solutions and in melts have involved mainly the use of NMR¹⁰⁻¹⁴ and radio-tracer techniques¹⁵⁻¹⁷. The results of the NMR studies are ambiguous, as it is difficult to separate the contributions to the overall motion due to translation of the molecule as a whole from those due to the internal (segmental) motion^{11,13}. McCall *et al.*¹⁰, for example, using the NMR

technique, have estimated the diffusion coefficient in molten polyethylene to vary as $D \propto M^{-5/3}$; their result, however, was based on rather limited data. In the case of the radio-tracer studies involving the variation of D with diffusant length in polymer melts, the results^{15,16} have been of a somewhat qualitative nature and have lacked a discriminative character.

In the present experiments the translational diffusion coefficient D of a series of deuterated linear polyethylene (DPE) fractions was measured in a protonated linear polyethylene (PPE) melt. Neutron scattering studies^{18,19} of polyethylene melts, in which small amounts of DPE were dissolved in PPE (and vice versa), indicate that the radius of gyration of the minor component in the melt is consistent with its having a random coil configuration. Thus if translational diffusion of the DPE molecules within the PPE melt is taking place by reptation, the relation of equation (1) must hold: and this is what I set out to test.

The measuring technique was developed by B. J. Briscoe and myself, and is based on infrared (IR) microdensitometry; it is described in detail elsewhere²⁰. In essence, the technique involves setting up a concentration step-function of the diffusant of interest within the matrix, and allowing it to broaden, with time, under diffusion. Measurement of the final diffusion broadened profile is carried out using an IR microdensitometer, which yields a value for D . The diffusant must absorb at some IR frequency unaffected by the matrix: in the present case, the labelling frequency was that of the C-D stretching mode (in DPE) at $2,170 \text{ cm}^{-1}$, a region free of absorption by the PPE.

Five fractions of linear DPE (98% deuterated) were used. Table 1 shows their molecular weight characteristics, as determined by gel permeation chromatography. The PPE matrix was linear polyethylene of $M_w = 1.6 \times 10^5$, $M_w/M_n \approx 15$ and $\text{CH}_2/1,000\text{C} < 1$; this molecular weight is much higher than the critical molecular weight for the onset of 'entangled' behaviour, as deduced from viscosity and other measurements^{1,2}. Solid solutions, 2% w/w, of each DPE fraction in the PPE matrix were prepared by dissolving and mixing both components in boiling xylene; the mixture was then precipitated from solution by pouring into cold excess methanol. After washing and drying, pellets were moulded from these precipitates and from pure PPE which had been similarly dissolved and precipitated, and these were coherently joined²⁰ to form composites containing a step function in DPE concentration (Fig. 2a). The composites were placed in suitable PTFE containers inside sealed glass ampoules under nitrogen, and left for various lengths of time at the diffusion temperature. On quenching (thereby effectively stopping further diffusion), slices were taken from the composites and scanned in the IR microdensitometer²⁰ to yield the diffusion broadened profiles (Fig. 2b).

Evaluation of D for a diffusion broadened step function is straightforward when dealing with a single diffusing species²¹. When a diffusant is not monodispersed, however, difficulties arise in interpreting the experimental data. This problem has led to serious anomalies in previous studies of self-diffusion, mainly with NMR techniques^{11,12}. In the present case, the final broadened profile (such as in Fig. 2b) is the sum of contributions from species of different molecular weight (within each DPE fraction, see Table 1), and hence different D values. If a diffusing species of molecular weight = M_i has a diffusion coefficient D_i associated with it, such that $D_i \propto M_i^{-2}$, then analysis shows (J.K. in preparation) that the slope of the broadened step function at the position of the original interface (Fig. 2b) is the experimental quantity which is to be measured. This yields an overall diffusion coefficient $\bar{D}$ associated with a diffusion-average molecular weight, M_D , such that

$$\bar{D} \propto M_D^{-2} \quad (2)$$

where

$$M_D = \left(\sum_i w_i M_i^{-2} \right)^{-1/2}$$

Characteristics were determined by gel permeation chromatography. N_w is the weight-average degree of polymerisation, corresponding to M_w .

Table 1 Molecular weight characteristics of the deuterated polyethylene (DPE) fractions.

Fraction	M_w	M_w/M_n	(N_w)
DPE1	3,600	2.25	(225)
DPE2	4,600	2.2	(288)
DPE3	11,000	3.4	(688)
DPE4	17,000	2.2	(1,063)
DPE5	23,000	1.8	(1,438)

w_i are the weight fractions of each species M_i comprising the diffusant sample.

In Fig. 3 $\bar{M}_w$ is plotted against the diffusion coefficient $\bar{D}$ as measured from the slope of the diffusion-broadened profiles such as in Fig. 2b. The best fit to the data is

$$\bar{D} \propto \bar{M}_w^p \quad (3)$$

where $p = -2.0 \pm 0.1$. But for $\alpha = -2$, M_D becomes identical with $\bar{M}_w$: for consistency of equations (2) and (3), therefore, α must be identified with p .

Thus for a DPE molecule of molecular weight M , diffusing in an 'entangled' PPE melt, the translational diffusion coefficient D scales as

$$D \propto M^{-2 \pm 0.1}$$

This result is to be compared with the reptation relation of equation (1); it may be contrasted with the relation $D \propto M^{-3.5}$ expected on the basis of, for example, Bueche's entanglement-coupling model.

These results provide direct experimental support for the suggestion that the translational diffusion mode of polymer molecules in 'entangled' melts is a curvilinear one. This has a number of implications.

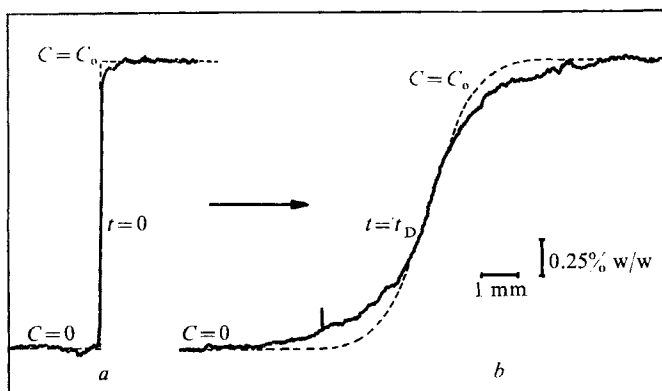


Fig. 2 Typical concentration profiles of deuterated polyethylene (DPE) in the protonated polyethylene matrix (PPE). *a*, Immediately subsequent to creation of a step-function. $C_0 = 2\%$ w/w of fraction DPE3 in PPE. The broken line represents an ideal step-function, and the continuous line is the profile as scanned in the infrared microdensitometer¹⁸. The scanning frequency is that of the C-D stretching mode at $2,170 \text{ cm}^{-1}$. *b*, The concentration profile of (*a*) following a diffusion run. Diffusion temperature $T_D = 176.0 \pm 0.3^\circ \text{C}$; $t_D = 1.114 \times 10^6 \text{ s}$; D as evaluated from the slope at the position of the original interface, is $3.7 \pm 0.6 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$. The broken curve is the theoretical Fickian profile for a single diffusing species with the diffusion coefficient D ; the deviations from the experimental profile are due to the polydispersity of the actual diffusant sample (see text and also equation (2)).

The effect of 'entanglements' on the slow relaxation modes in 'entangled' systems now seems to be clearer: the slowest of these modes corresponds to the centre-of-mass translational diffusion of the molecules, and is constrained to take place by reptation. The concept of localised entanglement coupling at a relatively small number of points, which lay at the heart of classical theory, must give way to the view where the effect of entanglements is one which pervades the length of a molecule: the net result is one of topological constraints on the motion of molecules. For sufficiently high molecular weight, curvilinear motion alone is allowed.

It is tempting to suggest, in view of these results, that the onset of snake-like motion corresponds also to the onset of entangled behaviour. This suggestion is explored elsewhere⁹. Within the range of experimental parameters described,

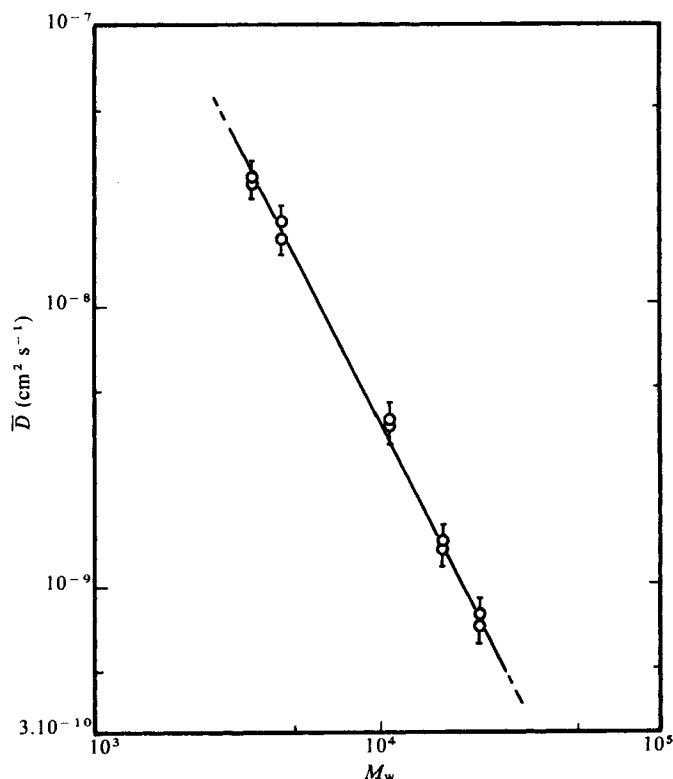


Fig. 3 The variation of $\bar{D}$ with $\bar{M}_w$ for deuterated polyethylene diffusing in a protonated polyethylene matrix, at a diffusion temperature of $176.0 \pm 0.3^\circ \text{C}$. Each point represents a separate experiment, and is the mean of 10 separate profiles such as in Fig. 2b. The lengths of experimental runs (t_D) vary by a factor of ~ 3 within each pair of experiments for a given DPE fraction. The least-squares best-fit to the data is the relation:

$$\bar{D} = 0.2 \bar{M}_w^{-2.0 \pm 0.1}, \text{ and is shown.}$$

however, the results clearly support De Gennes' notion of reptation in an entangled polymer system.

I thank Professor Sir S. F. Edwards for stimulating discussions, Dr D. G. Ballard and Dr G. Longman for supplying the deuterated fractions, and Professor D. Tabor and Dr B. J. Briscoe for their encouragement. A grant from Dow Corning Chemicals and a Research Fellowship from St Catharine's College, Cambridge, are both gratefully acknowledged.

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Improving the timbre and responsiveness of a bass trombone

THE design of brass instruments has always been a largely heuristic procedure. Recently, however, a knowledge of the acoustic impedance of the instrument has been used to correct faulty intonation in a trumpet¹. Here we demonstrate how such knowledge may be used to improve both the timbre and the responsiveness (or 'feel') of a bass trombone.

Bass trombones commonly use one or more valves (in addition to the slide) to increase the length of the instrument, thereby reducing the playing frequency. The instrument used in this experiment possesses a 'G valve' which lowers the pitch of a Bb instrument by three semitones. It follows, therefore, that the note G2 (98 Hz) may be played in two ways, either (1) the slide in 1st position and the valve depressed; or (2) the slide in 4th position and the valve released. In both cases the overall length of the instrument is the same, but the extra tubing is introduced at different points. J.M.B. noted that using the valve to play G2 resulted in a note that was difficult to sustain comfortably, and that this difficulty further manifested itself in a marked deterioration of the timbre.

The acoustic impedance of the instrument with all its tuning slides fully in was measured for both of the above cases, using apparatus described elsewhere². The results are shown in Fig. 1. The plots are similar, but the fundamental of G2 (which is the second maximum) is flatter by 6 Hz using the valve. There are also differences between the amplitudes of the maxima, but we shall confine ourselves to matching the frequencies of the fundamental as closely as possible for the two cases. The need to do so is explained by Benade³. Since the sound generating mechanism of brass instruments is non-linear, it may be shown that all the maxima which lie close to integral multiples of the playing frequency interact to sustain a note to an extent depending on the playing dynamic. This interaction is referred to by Benade as a "regime of oscillation". When using the valve to play G2 the fundamental is too flat for a truly successful 'regime' although the remaining maxima are reasonably well positioned. What is required then, is to raise the frequency of the fundamental when the valve is used without altering the positions of higher maxima.

Wogram⁴ introduces the concept of a 'sum function' which he calculates by determining the real part of the input impedance, and then summing the input resistance values at harmonics of a given fundamental frequency. He further shows that the peaks of this 'sum function' are simply related to the playing frequencies of an instrument,

Fig. 1 The acoustic impedance of the instrument before modification. Using the valve (dashed line); not using the valve (solid line).

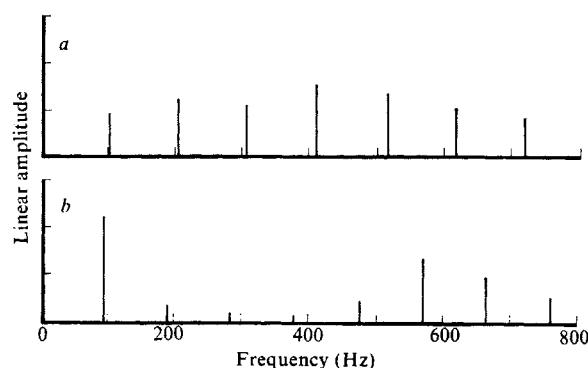
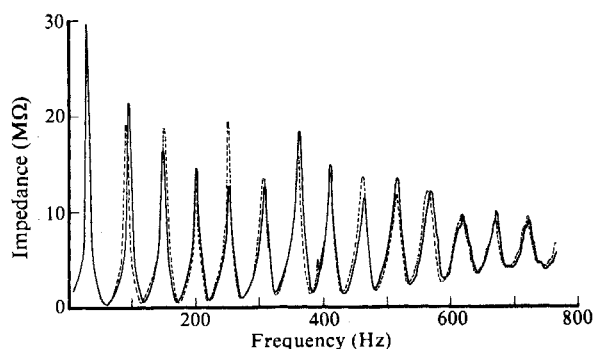


Fig. 2 The internal spectra of the modified instrument *a*, ff; *b*, pp.

and thus enable one to study the way the playing frequency changes with playing dynamic in a systematic manner. We have also written a computer program which calculates the internal spectrum for any playing frequency but in particular the ones predicted by the peaks of the 'sum function'. When the 'sum function' is calculated for the bass trombone using the valve it is found that at high dynamic levels the playing frequency is 103 Hz but at low levels it falls to 94 Hz and the internal spectrum envelope deteriorates considerably (see Fig. 2). Interestingly the change of playing frequency also appears when 4th position is used, though to a smaller extent, indicating that the possibility still remains of affecting further slight improvements to the whole instrument.

A perturbation theory exists to move the positions of the resonances of the trumpet¹ and a simple scaling procedure enables it to be used for a trombone. Clearly it is necessary to restrict the changes only to the G valve section so that the parts common to the Bb and G instruments are unaffected. Calculations indicated that a 9% reduction in the cross-sectional area of the tube forming the G valve was required. A new section was, therefore, fitted, and the acoustic impedance was again measured. The results indicate a considerable improvement although the fundamental is still 3 Hz too flat.

To determine whether any real subjective improvement had been made, a professional bass trombone player was invited to compare this instrument with an unmodified one whose impedance curve was measured and found to be identical (within experimental error²) to the results shown in Fig. 1. The player wore a blindfold and heavy gloves during the experiment, and the slides of both instruments were cleaned and oiled so that they felt as similar as possible. (This precaution is necessary because musicians are strongly influenced by the slide quality⁵.) The player was presented with a pair of instruments sequentially. Each pair was either a repetition of a given instrument or the two different instruments. The player was then instructed to play the note G2 under both conditions (with and without the valve) and then state whether he thought they were the same or different. If he thought they were different he was then asked which one of the pair he preferred. During extensive trials he could always distinguish between the two different instruments, and always correctly identified the repetitions. He referred to the unmodified instrument as "stuffy", "dull", and "hard to play" when using the valve. He greatly preferred the modified instrument and stated that the two playing conditions were "as closely matched as possible".

Thus the timbre and responsiveness, in addition to the intonation, may be improved given an apparatus for measuring acoustic impedance, and a theory which relates the geometry of the instrument to the frequency distribution of the impedance maxima.

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Guinea Current upwelling

THE historical record of maritime observations are summarised here to indicate the dominant seasonal variations in upwelling, and in certain associated processes, within the Guinea Current region. The region is similar to other eastern ocean boundary upwelling areas in the appearance of cool sea temperatures near the coast, productive coastal fisheries, and a zone of low rainfall on the adjacent coast (Fig. 1). It differs from some of the more studied regions in several important respects. These include the zonal rather than meridional trend of the coast, the influence of a rather narrow intense coastwise current, and an unusual lack of correspondence on the seasonal time scale between sea-temperature features attributable to upwelling, and features in the overlying wind stress field¹. There seems to be a link between interyear variations in upwelling intensity and corresponding variations in both coastal rainfall and local fishery success.

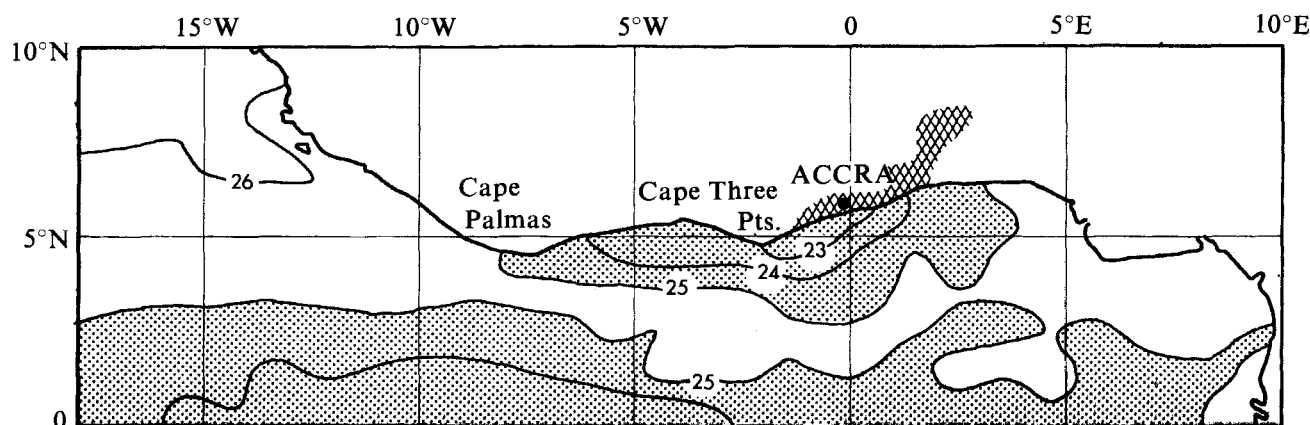
To construct the sea-temperature distribution shown in Fig. 1, maritime reports for the period 1850–1970 were obtained from the U.S. National Climatic Center's file of marine surface observations (TDF-11), checked for gross errors, and summarised by month and by 1° 'square' areas. Mean values were contoured by hand, taking into account the abundance of observations and magnitude of standard deviations. Data abundance was greatest near the coast and to the west of Cape Palmas. The effect of warm advection in the eastward-flowing Guinea Current is apparent in the shape of the 26 °C isotherm and in the offshore temperature

maximum which separates the cool temperatures along the coast east of Cape Palmas, attributable to coastal upwelling, from cool temperatures near the equator which are probably related to equatorial upwelling.

The seasonal distribution of sea-surface temperature within a line of 1° square areas stretching southward from the coast near Cape Three Points (Fig. 2) features an offshore maximum every month, suggesting continuation of some degree of coastal upwelling throughout the year. The offshore gradients are most intense from June through October; coolest coastal temperatures are reached during August and September. A secondary coastal minimum appears during January, resulting in a two-peaked annual temperature cycle.

A similar display of the seasonal cycle of sea-surface temperature (Fig. 3), this time within a line of 1° square areas arranged along the coastal boundary, reveals continuity of the summer minimum along the entire north coast of the Gulf of Guinea. In Fig. 3, west is towards the top (see Fig. 3d); thus the direction of flow of the Guinea Current is from the top towards the bottom of the diagram. The temperature decreases in the direction of flow to an absolute minimum east of Cape Three Points. Although the contour interval in Fig. 3 is too coarse to show it, much of the temperature decrease occurs in two abrupt steps upon passing each of the two major capes, Cape Palmas and Cape Three Points. Particularly sharp temperature gradients have been reported off Cape Palmas². The coolest region, defined by the 24 °C isotherm, appears in Fig. 3 to be split into two lobes by slightly higher temperatures from 0–1°W longitude; this is an artefact due to that particular 1° square being displaced slightly offshore relative to the squares to either side. Eastward of this late summer minimum feature, sea-surface temperature increases towards a region increasingly affected by outflow from the Niger River Delta and other sources. A cold temperature feature is apparent during winter in the extreme western portion of the region. The sharp gradient near 15°W longitude indicates the southern limit of the tropical front which oscillates seasonally along the west African coast between 10°N and 20°N latitudes, apparently responding to seasonal variations in wind-induced upwelling and associated along shore advection³. Extending eastwards from the front is a winter temperature minimum which retains its identity along the Guinea Coast well into the Bight of Biafra (~7°E longitude) where it is evident as a slight reversal during January. The two seasonal temperature minima are separated by periods of relatively warm sea-surface temperatures, with the major maximum occurring during spring.

Fig. 1 Long-term mean distribution of sea-surface temperature, summarised by 1° square areas for August. The contour interval is 1 °C; temperatures less than 25 °C are shaded. The crosshatched area on the coast in the vicinity of Accra indicates an area of less than 40 inches annual rainfall, and defines the approximate extent of the Accra dry belt¹⁶.



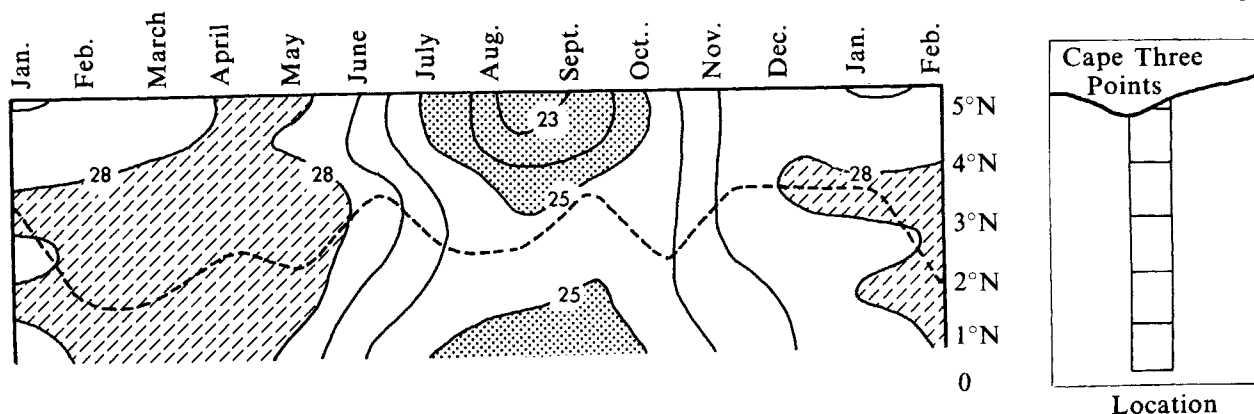


Fig. 2 Long-term composite annual cycle of sea-surface temperature within the line of 1° squares normal to the coast near Cape Three Points shown to the right. The contour interval is 1°C ; temperatures less than 25°C are shaded, and those greater than 28°C are indicated by broken diagonal hatching. The location of the maximum mean temperature within the line of squares for each long-term monthly sample is indicated by the dashed line.

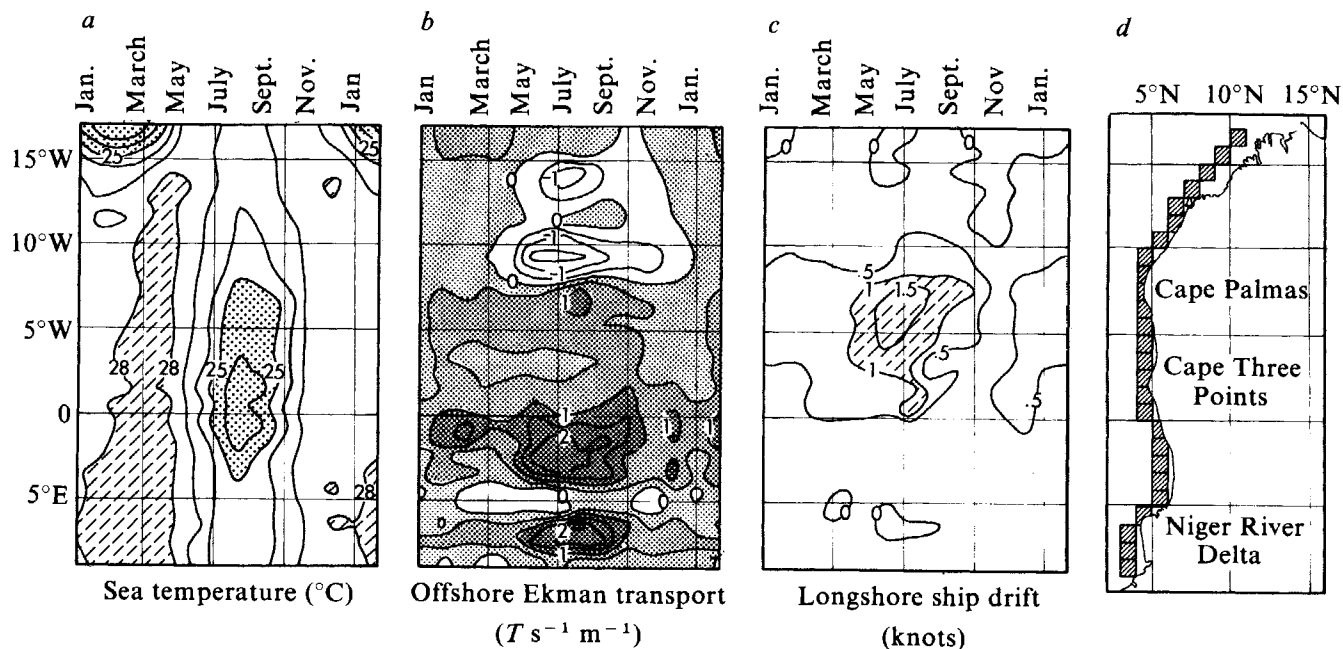
Being near the equator, the Guinea Current region experiences two periods of vertical sun per year, one shortly before the vernal equinox and the other shortly after the autumnal equinox. Unlike the situation in more temperate latitudes, both solstices are periods of low sun. Thus, the double peaked seasonal cycle of sea temperature matches qualitatively the cycle of solar height. But compilations of mid-ocean sea-surface temperatures⁴, at similar latitudes where the cycle of solar height is identical, indicate no such strong correspondence. Thus, it is difficult to ascribe the observed features directly to such large scale effects as passages of the Sun. Rather, they would seem to be almost entirely controlled by processes local to the Guinea Current region.

In the classical model for coastal upwelling⁵, water set in motion by the stress of the wind is deflected offshore by the rotation of the Earth. When this occurs along an extensive stretch of coast such that the water transported off

the coast cannot readily be replaced by convergence of horizontal alongshore flow, replacement can occur by upwelling of deeper water.

Previous comparisons of offshore Ekman transport distributions with corresponding temperature distributions in the California Current⁶, and Canary Current⁷ regions on a similar 1° scale have yielded general conformities in major features, indicating seasonal variation in the local wind as the principal factor controlling seasonality in the coastal upwelling process. Accordingly, wind reports from the Guinea Current region were assembled from the same source as the temperature reports. These were converted to measures of sea-surface stress by squaring each reported wind speed and multiplying by the density of air (assumed constant, 0.00122 g cm^{-3}) and a constant drag coefficient (0.0013). The derived stress 'reports' were then averaged by components to yield a resultant mean stress for each long term composite month and 1° square area. This was con-

Fig. 3 Long-term composite monthly variations within the 1° square areas arranged along the coast, shown in (d). a, Sea-surface temperature. The contour interval is 1°C ; temperatures less than 25°C are shaded and those greater than 28°C are indicated by broken diagonal hatching. b, Offshore-directed component of Ekman transport. The contour interval is 0.5 tonnes S^{-1} transported across each 1 m width. Positive values, indicating seaward transport, are shaded; darker shading indicates more intense seaward transport. c, Alongshore component of ship drift. The positive direction is such that the coast is on the left when facing downstream. The contour interval is 0.5 knots ; speeds greater than one knot are indicated by broken diagonal hatching.



verted to Ekman transport, directed 90° to the right of the stress vector, by dividing by the Coriolis parameter computed at the mid latitude of the 1° square. Where the centre of the square is less than 5° from the equator the value of the Coriolis parameter at 5°N latitude was used. Offshore components were resolved along characteristic offshore normal directions, based on the nearshore bathymetry within each individual square.

The seasonal pattern of offshore-directed Ekman transport (Fig. 3) reveals certain features which correspond to features in the temperature pattern. Transport tends to be offshore throughout the year, except during certain months off Liberia and Sierra Leone ($8\text{--}14^\circ\text{W}$) and near the west-facing portion of the Niger River Delta shoreline ($4\text{--}6^\circ\text{E}$). A winter maximum of offshore transport occurs in March at the northwestern end of the area (top of the diagram), and retains continuity toward the east, shifting to February off Cape Palmas and Cape Three Points. This winter maximum generally corresponds to the winter temperature minimum described above. During the summer there is a relative maximum of offshore Ekman transport on the east side of Cape Palmas and another increase to the east of Cape Three Points; these correspond to the major decreases in sea temperature near these capes.

Maximum values of offshore transport occur to the east, however, that is downstream in relation to Guinea Current

observations produced as part of this study show a very significant component of the mean wind to be directed onshore at all seasons. Any larger portion of the transport being directed with the wind would, therefore, only imply a further reduction in local wind forcing of upwelling at the coast.

Figure 3c summarises reports of drift attributed to ocean currents compiled from ship's logbooks by the U.S. Naval Oceanographic Office. Although the number of available reports is very small, being of the order of 20 per month-square in the area from Cape Palmas to Cape Three Points, a coherent picture of the seasonal cycle of flow in the Guinea Current is apparent. Off Cape Palmas the current near the coast intensifies dramatically, diminishes slightly toward Cape Three Points, and drops off rapidly past Cape Three Points. Mean velocities near Cape Palmas vary from nearly 2 knots in July to less than half a knot in November. The current maximum is thus somewhat upstream of and slightly preceding the summer temperature minimum, actually coinciding in time to the period of most rapid temporal decrease of temperature and in space to the zone of most rapid spatial decrease. Horizontal advection in the current, of course, opposes the temperature decrease. Various mechanisms leading to vertical advection in an intensified current⁷⁻¹⁰ could perhaps contribute to the observed cooling. But, the lag of at least a month between

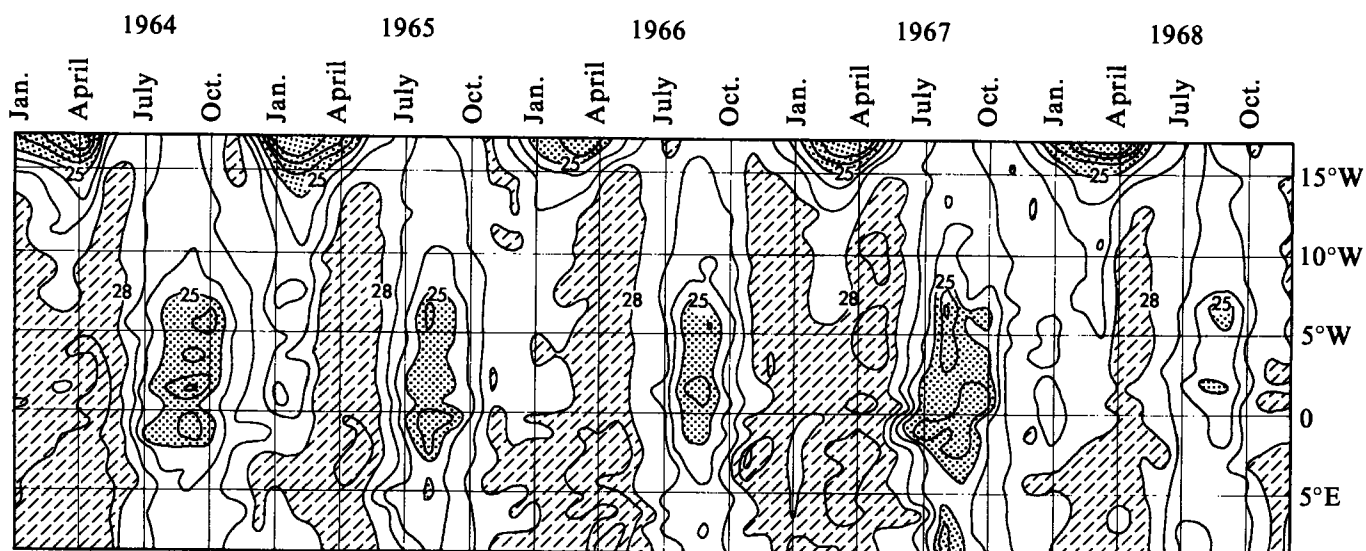


Fig. 4 Monthly temperature variation within the square areas shown in Fig. 3, during the years 1964 through 1968. The contour interval is 1°C ; temperatures less than 25°C are shaded and those greater than 28°C are indicated by broken diagonal hatching.

flow, of the location of summer minimum temperatures. The offshore transport maximum off Dahomey ($2\text{--}4^\circ\text{E}$) is in the area of most rapid down-stream warming. Likewise, a second offshore transport maximum in the Bight of Biafra ($6\text{--}9^\circ$) is not strongly reflected in the sea-temperature distribution, although both cases may involve some masking of the effects of upwelling by low salinity surface water. Certainly, there is nothing in the local Ekman transport distribution to account for the continuity of the temperature minimum to the west, that is, upstream, of Cape Palmas.

Since this region is very near the equator, the diminished Coriolis term in the dynamical equations may lead to a different balance of forces from that assumed in the Ekman transport relationship. In such a case the transport could be at a lesser angle than 90° to the direction of wind stress. Such an effect, however, cannot explain the non-correspondence of the distributions in Fig. 3. Summaries of wind

the maximum current speed in July and the minimum sea-surface temperatures in August–September (Fig. 3) is difficult to reconcile with any predominant effect of such processes; for example, at the indicated average current velocities, water upwelled near Cape Palmas during the July current maximum would be carried past Cape Three Points in less than 10 days.

Recent theoretical developments have indicated the ability of upwelling-produced displacements of the internal density structure to propagate in the form of coastal trapped waves, and thus to affect the temperature and current velocity fields at other locations along the coast^{11,12}. In the configuration of the Guinea Current region, the direction of propagation would be to the west. The degree of westward displacement of features in the sea temperature and drift patterns, compared to features in the Ekman transport field (Fig. 3), is consistent with computations from theory

Table 1 Comparison of intensity of summer-fall upwelling with July–October rainfall at three reporting stations in Ghana and with catch of the Ghanaian herring fishery for the years 1964 through 1968

		1964	1965	1966	1967	1968
Upwelling		High (late)	Intermediate	Intermediate	High	Low
Month		J A S O*	J A S O	J A S O	J A S O	J A S O
Rainfall (quintiles)	Accra	3 1 0 0	5 5 3 2	4 3 1 3	1 2 5 1	6 6 6 5
	Takoradi	2 4 1 0	4 4 5 1	5 4 2 2	2 2 3 1	6 6 6 5
	Kumasi	3 4 1 2	4 5 3 5	6 5 1 2	2 1 2 1	6 6 6 1
Summation		21	46	38	23	65
Herring catch (Ghana)		34.2	7.8	13.3	43.2	12.2

Upwelling intensity is estimated from sea-temperature distributions (Fig. 4). Rainfall is given in quintiles of the frequency group within which the recorded precipitation falls relative to a group of reference years¹⁷. 0 indicates monthly precipitation to have been lower, and a '6' indicates it to have been higher, than in any corresponding month in the reference series. In the line labelled summation the quintile values for the four months and the three stations are added together to give a seasonal index. The herring catch is in 10³ tonnes.

*J, July; A, August; S, September; O, October.

(A. J. Clarke, personal communication). Thus it seems that such processes may be exerting particularly strong control on the pattern of seasonal upwelling in the Guinea Current.

In the surface marine observation file (TDF-11) there is an abrupt increase in the density of reports in the Guinea Current region beginning with 1964. In the particular version of the file that was used in this study, however, the frequency begins to drop off rapidly after 1968. For these five years of maximum data density a time series of sea-surface temperature (Fig. 4) was constructed on the same basis as the long-term composite annual cycle (Fig. 3). Because of the higher short-term variance of the wind, the data density was not sufficient to derive a meaningful similar series of wind or Ekman transport variations.

If one examines the summer upwelling feature (10°W–5°E longitude) in Fig. 4, it is possible to class the summers of 1964 and 1967 as having been cold (strong upwelling), 1968 as having been warm (weak upwelling), and 1965 and 1966 as having been intermediate (Table 1). In addition the strong upwelling in 1964 seems to have occurred relatively late, the coolest monthly mean temperatures appearing in September rather than in August as is the case in long-term composite cycle (Fig. 3). In partial corroboration of this classification, dissolved oxygen data collected off Ivory Coast (~4°W) during the period 1966–70¹³ show low oxygen values characteristic of deeper layers unmistakably nearer the surface during the summer upwelling season of 1967 than in the other years of that particular series.

With these upwelling estimates, it is possible to look for apparent effects of upwelling variations on coastal rainfall. One mechanism which may contribute to the intense coastal aridity, which is a general characteristic of upwelling regions, is the stabilisation of the shoreward airflow by the cool upwelled surface waters, thereby inhibiting vertical development of storm clouds¹⁴. The Accra dry belt (Fig. 1), which has the appearance of a coastal enclave of semi-arid savanna surrounded by tropical rainforest, has been termed 'the most remarkable climatic anomaly of the Guinea coastlands'¹⁵. One of the unusual features cited is an anomalous dearth of rainfall during the late summer. Prevailing winds are from the south-west¹⁶; Figure 1 shows the Accra dry belt to be oriented directly downwind of the coldest sea-surface temperatures.

Table 1 classifies the rainfall recorded at three locations in Ghana¹⁷. Takoradi is located on the coast, just east of Cape Three Points. Kumasi is about 100 miles inland, between Takoradi and Accra. The correlation of the rainfall record with the upwelling estimates is convincingly strong. During the years of strong upwelling, 1964 and 1967,

the July–October rainfall was anomalously low; in fact 'late' upwelling estimated for 1964 corresponds to 'late' rainfall deficits in September and October. During the weak upwelling year, 1968, monthly mean rainfall actually exceeded the highest class intervals established from historical data at all three locations during the months of July, August, and September.

Also listed in Table 1 are the landings of herring (*Sardinella aurita*) in Ghana during these same five years¹⁸. This was Ghana's most important fishery during this period, largely operating from canoes very near shore during the summer-fall upwelling period¹⁹. Evidently periods of extremely good fishing were associated with strong upwelling, as are periods of subnormal coastal rainfall. During these years a sharply increased trend in fishing effort occurred, due to technology and so on. If this is taken into account in interpreting the landing figures, a relationship is even more apparent.

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The origin and relative abundances of C, N and the noble gases on the terrestrial planets and in meteorites

RASOOL and Le Sergeant¹ adopted the inhomogeneous accretion model for planets discussed by Turekian and Clark² and considered the question of whether carbonaceous chondrites or LL chondrites could better explain the relative abundances of volatile elements found on Earth and Mars. In the inhomogeneous accretion model the volatile elements of the terrestrial

planets are attributed to a component which is added late in the accretional history of the planet. Turekian and Clark² presumed this late-stage component to be like carbonaceous chondrites. But, Rasool and Le Sergeant¹ compared the relative concentrations of H, C, N, Ne, Ar, Kr, and Xe (normalised to C) for the Earth, C1 (carbonaceous) chondrites, and LL (ordinary) chondrites and concluded that relative volatile abundances for the Earth have greater similarity to LL chondrites than to C1 chondrites. Specifically, these authors noted that relative to carbon, abundances of the noble gases (Ne, Ar, Kr, and Xe) are within a factor of 3.8 for the Earth and LL chondrites, but differ by factors of 15–37 between the Earth and C1 chondrites. Abundances of H and N relative to C were indicated to be the same within a factor of 2.5 for both meteorite types and the Earth. Turekian and Clark³ had previously made similar comparisons between the Earth and C1 chondrites and concluded that terrestrial volatiles were acquired from material having H/N/C ratios similar to C1 chondrites, but with order-of-magnitude higher abundances of the noble gases. Rasool and Le Sergeant, on the other hand, concluded that volatiles on Earth and, by extension of their arguments, also volatiles on Mars can better be explained by late accretion of material similar to LL chondrites rather than material similar to C1 chondrites. We disagree with the conclusion reached by Rasool and Le Sergeant and with the LL-chondrite data which they used to reach their conclusion. Our purpose here is to demonstrate (1) that the noble gas/C and noble gas/N ratios presently determined for the Earth differ by an order of magnitude from these ratios in most meteorites; (2) that as a group LL chondrites show no better agreement with the Earth in these ratios than do C1 chondrites; (3) data are too incomplete to lead to any conclusions about the type of meteoritic material which may have supplied volatiles to Mars. In addition we suggest that the noble gas/C and noble gas/N ratios for Venus are likely to be more like the Earth's than like meteorites.

Figure 1 plots ^{36}Ar concentrations against the $^{36}\text{Ar}/\text{C}$ ratio given in units of $10^{-8} \text{ cm}^3 \text{ per g weight } \%$ for several types of meteorites. The major trend is for the $^{36}\text{Ar}/\text{C}$ ratio to decrease as the ^{36}Ar concentration decreases, which probably reflects either different formation temperatures, or the greater ease by which ^{36}Ar can be lost from material compared to C. The LL chondrites of petrographic grades 4–6 have ^{36}Ar concentrations two orders of magnitude lower and $^{36}\text{Ar}/\text{C}$ ratios a factor of ~ 1 –3 lower than carbonaceous chondrites. Other ordinary H and L chondrites of the same petrographic grades have very similar ^{36}Ar and C contents to the LL chondrites shown. Unequilibrated chondrites (ordinary chondrites of petrographic grade 3) have intermediate ^{36}Ar concentrations and seem to show the greatest range in $^{36}\text{Ar}/\text{C}$ ratios among the various meteorite types. But, the two highest $^{36}\text{Ar}/\text{C}$ ratios shown for unequilibrated chondrites (Krymka at 185 and Chainpur at 124) must be considered dubious, as separate ^{36}Ar analyses on these meteorites showed variations up to a factor of 10. A separate analysis of Chainpur gives the data plotted at $^{36}\text{Ar}/\text{C} = 20$. Ureilites, whose ^{36}Ar is associated with diamond and graphite, have the highest ^{36}Ar concentrations of any meteorite type. The two Murchison (C2) data points are acid-etched, nonsilicate residues containing $\sim 50\%$ organic polymer C, but comprising only ~ 1 –2% of the total meteorite. Several analogous acid-etched residues from Allende (C3) showed amorphous C contents of ~ 18 –97% and $^{36}\text{Ar}/\text{C}$ ratios of ~ 2 –10, with one value of 97 (ref. 4). No substantial data exists which indicates $^{36}\text{Ar}/\text{C}$ ratios in meteorites higher than $\sim 100 \times 10^{-8} \text{ cm}^3 \text{ per g weight } \%$.

In comparison to the meteorite data in Fig. 1, the ^{36}Ar concentration and $^{36}\text{Ar}/\text{C}$ ratio for the whole Earth have been estimated as $2.1 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$ and 473 ($10^{-8} \text{ cm}^3 \text{ per weight } \%$) respectively², and plot far off Fig. 1 to the right. The $^{36}\text{Ar}/\text{C}$ ratios for the Sun⁴ and the atmosphere of Mars⁵ are larger than this ratio for the Earth by factors of 5×10^5 and 23, respectively. Individual meteorites have $^{36}\text{Ar}/\text{C}$ ratios lower by factors of 5–100 relative to the Earth. Considering the average $^{36}\text{Ar}/\text{C}$ of different classes of meteorites compared to the Earth, C1s are

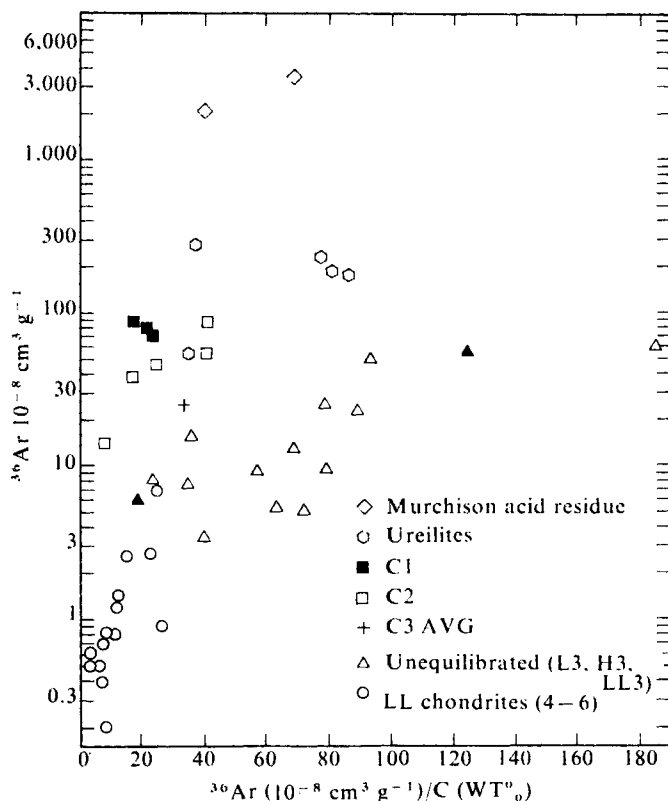


Fig. 1 Relationship between ^{36}Ar concentrations and $^{36}\text{Ar}/\text{C}$ ratios for several meteorite types. Units for $^{36}\text{Ar}/\text{C}$ are $10^{-8} \text{ cm}^3 \text{ per g weight } \%$, and are the typical analytical units reported for these elements. Each data point represents a separate meteorite except the two acid residue points for Murchison²⁷, two points for Chainpur LL3 $\blacktriangle$, and the averaged value for C3 chondrites. On this plot the Earth has a $^{36}\text{Ar}/\text{C}$ ratio of 473 and a ^{36}Ar concentration of 2.1 (ref. 2) and would plot far off the figure to the right. Meteorite data are taken from refs 7, 9, 10, 22, 25, 26.

lower by a factor of ~ 20 (as previously discussed²), C2s and C3s by a factor of ~ 14 , ureilites and unequilibrated chondrites by a factor of ~ 10 , and ordinary (LL) chondrites by a factor of ~ 45 . Ratios of Xe/C and Kr/C in meteorites are also lower than those ratios for the Earth by an order of magnitude or more. This conclusion arises from the fact that proportions of planetary Ar/Kr/Xe in various meteorite types are relatively constant, generally within a factor of 5 for Ar/Xe and a factor of 3 for Kr/Xe (refs 6–8), over a concentration range of three orders of magnitude. If anything ratios of Ar/Xe and Ar/Kr increase slightly with increasing ^{36}Ar concentration.

Neon in meteorites is more difficult to compare as it does not usually correlate with Ar. Neon in ordinary chondrites is almost entirely produced by cosmic ray interactions, and measurable amounts of planetary Ne has rarely been reported in these meteorites. Many individual meteorites representing essentially all meteorite types contain large quantities of neon implanted by the solar wind, and therefore are ill-suited for a comparison of planetary volatiles. Neon in ureilites is mainly produced by cosmic ray interactions, and the $^{20}\text{Ne}/^{36}\text{Ar}$ planetary ratio in ureilites is probably ≤ 0.01 (refs 7, 9). The unequilibrated chondrites also seem to contain little planetary Ne¹⁰. Even in carbonaceous chondrites the $^{20}\text{Ne}/^{36}\text{Ar}$ ratio varies by two orders of magnitude (~ 0.2 to 20) and indicates the presence of appreciable amounts of solar Ne in many samples⁷. The best estimate of the planetary $^{20}\text{Ne}/^{36}\text{Ar}$ mole ratio in carbonaceous chondrites is 0.28 (ref. 7), compared with the Earth value of 0.53 (ref. 2). With these values the $^{20}\text{Ne}/\text{C}$ ratio would be ~ 6 in carbonaceous chondrites and ~ 250 for the Earth. This is a difference of ~ 40 , which is essentially the value adopted by Turekian and Clark².

Available data, therefore, do not support the conclusion by Rasool and Le Sergeant¹ that the ratios of noble gases to carbon on Earth are much more similar to those of LL chondrites. The ³⁶Ar and C concentrations of LL chondrites, including the unequilibrated L3 chondrites, vary by factors of ~500 and ~22, respectively^{12, 13}. Thus the use of average or median concentrations from different populations instead of determinations for specific meteorites can be misleading. These authors reference the source of their noble gas and C data as two review papers. We surmise from their calculated ratios and from references given that they adopted a median C concentration of LL-chondrites¹² and an average noble gas abundance, which is also similar to a specific determination listed for Chainpur¹³. Chainpur is an LL chondrite which happens to be unequilibrated, and consequently has higher ³⁶Ar concentration and ³⁶Ar/C ratio than typical LL chondrites (Fig. 1). Various measured ³⁶Ar values for Chainpur also differ from one another and are lower by a factor of 4–20 than the LL chondrite ratio used by Rasool and Le Sergeant¹. The average ³⁶Ar/C of LL chondrites of petrographic grades 4–6 (Fig. 1) would be a factor of 45 lower than the ratio adopted by these authors. Similarly, the ³⁰Ne/C ratio adopted by Rasool and Le Sergeant for LL chondrites is much too high. As pointed out above, LL chondrites and unequilibrated chondrites (including Chainpur whose Ne content was apparently used by Rasool and Le Sergeant) rarely contain measurable amounts of planetary Ne (see, for example, refs 6, 10).

abundance pattern of volatiles on Earth could be derived from Ar-rich meteorites such as C1 chondrites by simple fractionation, as this would require greater loss of C relative to Ar, Kr, and Xe, and could be expected to fractionate Ar/Kr/Xe. The possibility of deriving the terrestrial volatiles from a component from which C had been selectively scavenged seems inconsistent with the relative nearness of the Earth to the Sun.

Rasool and Le Sergeant¹ also compared the relative abundances of volatile elements on Mars with their calculations for LL chondrites. Instead of normalising elemental ratios to C, for which the nonatmosphere inventory is unknown, they normalised to ³⁶Ar. They noted that the Kr/Ar and Xe/Ar ratios differed by only a factor of 3 between Mars volatiles and their LL chondrite values, and they used this similarity as evidence that volatiles on Mars were also introduced by a carrier similar to LL chondrites. But, such an argument based on the observed similarity in Kr/Ar and Xe/Ar ratios is trivial, because, as we summarised earlier, the Ar/Kr/Xe ratio is nearly constant for planetary gases in ordinary chondrites, unequilibrated chondrites, carbonaceous chondrites, ureilites, and the Earth. Thus, nearly constant Ar/Kr/Xe ratios would result no matter which of the above materials carried volatiles to Mars.

Another comparison is to consider the ³⁶Ar/N/C atom ratios in the atmospheres of Mars and Venus with these values for Earth and the meteorites (Table 1). The C and N abundances for meteorites and the Earth are measured and estimated values,

Table 1 Atom ratios of volatile elements in terrestrial planets and meteorites

Object	Ref.	N/C × 10 ⁻²	³⁶ Ar/N × 10 ⁻⁶	³⁶ Ar/C × 10 ⁻⁶	K/C	³⁶ Ar/K × 10 ⁻⁶
Earth*	2,21	4.6	5.5	0.25	1.8	6.3
Mars†	5	5.3	110	5.7	?	?
Venus‡	18	3.6	?	(200)‡	?	?
C1 chondrites§	7,22–24	3.7	0.33	0.012	0.0044	280
Ordinary chondrites	23–26	0.9–5	0.1–0.7	0.005	0.25	2.2

*K content of the Earth (minus the core) is taken as one-half chondritic²¹.

†Data for Mars and Venus are for atmospheres only. Reported data for Mars⁵ are assumed to be in volume %.

‡Total Ar/C ratio, where total Ar = ⁴⁰Ar + ³⁶Ar.

§N values used for C1s are recent measurements²³, and are approximately a factor of 2 lower than previous values².

||N abundances in ordinary chondrites show large variations^{22, 23, 25, 26}, and consequently only a range in N/C and ³⁶Ar/N is given.

What possible explanations exist for the observation that the Earth has considerably higher noble gas/C and noble gas/N ratios than known meteorite types (Fig. 1)? (1) Such ratios estimated for the whole Earth² may be incorrect. It is very unlikely that the abundances of Ar, Kr, and Xe for the Earth have been overestimated since the atmosphere contains the bulk of these gases, and the atmospheric abundances are known to a relatively high accuracy. The converse explanation, that the estimated abundances of C and N in the whole Earth² may be too low by a factor of 5–50, is perhaps possible. Essentially all of the estimated C in the Earth² is present as C in sediments (33 %) and ultramafic (mantle) rocks (~66%). Approximately 28 % of the estimated N in the Earth, on the other hand, is present in the atmosphere², and is known to a relatively high accuracy. (2) The Earth may have acquired its volatiles from a source unlike known meteorite types and one with higher Ar/C and Ar/N ratios. This possibility is plausible since the oxygen isotopic abundances of terrestrial materials is distinct from those of ordinary chondrites, carbonaceous chondrites, or ureilites¹¹. Unfortunately, Ar/C ratios are not known for differentiated objects (for example, achondrites, mesosiderites and the Moon) which have oxygen isotopic patterns analogous to the Earth¹¹, but which contain very little planetary Ar. (3) It seems unlikely that the relative

respectively, for the whole bodies, whereas the C and N abundances for Mars and Venus are the atmosphere inventories only. The ³⁶Ar/C and ³⁶Ar/N ratios for Mars are higher than those of Earth and meteorites by factors of ~20–4,000, which undoubtedly results because only a small fraction of the C and N acquired by Mars currently resides in its atmosphere⁵. Based on their assumption that volatiles on Mars have abundances like their calculated LL chondrite values, Rasool and Le Sergeant¹ estimated that ~3.5 % of the original C and ~6 % of the original N on Mars would be present in its atmosphere (assuming that all of the original ³⁶Ar is present in the atmosphere). If known meteorite types (Fig. 1) contributed the volatile elements to Mars, however, these values must be lowered, and only ~0.05 % of the original C and ~0.3 % of the original N on Mars would be present in its atmosphere. These values are consistent with the suggestion that large amounts of carbonates may reside in surface materials¹⁴, and with the estimate made from the ¹⁵N/¹⁴N ratio measured by Viking that ~10–150 times as much N has been lost to space from the atmosphere of Mars as has been retained¹⁵. It is interesting to note that the N/C ratio varies only little among the different objects listed in Table 1 in spite of variations in ³⁶Ar/N and ³⁶Ar/C by two orders of magnitude and more. Even the N/C ratio for the Mars atmosphere, which

apparently has lost large fractions of C and N by different mechanisms, is within a factor of 2 of the N/C values for the whole Earth and meteorites. The N/C ratio, therefore, is not likely to be diagnostic of any component which may have contributed volatiles to the terrestrial planets.

As discussed above, neither the relative abundances of volatile elements supplied to Mars, nor the fractions of original volatiles which now reside in its atmosphere can be precisely determined from the present data. This statement also applies to conclusions drawn from the measured $^{40}\text{Ar}/^{36}\text{Ar}$ ratios, as neither the $^{36}\text{Ar}/\text{K}$ or K/C ratios nor the fraction of ^{40}Ar degassed into the Martian atmosphere are known. It has been suggested that the high $^{40}\text{Ar}/^{36}\text{Ar}$ ratio for the Mars atmosphere ($2,750 \pm 500$ (ref. 5)) compared with the Earth's atmosphere (296) may be due to a higher K content and $\text{K}/^{36}\text{Ar}$ ratio for Mars compared with the Earth, or that ^{36}Ar has escaped from Mars^{16,17}. Fanale¹⁴ has suggested that both Earth and Mars received a K content similar to chondrites and that Mars received an ^{36}Ar content at least as high as Earth's. The K/C , K/N , $^{36}\text{Ar}/\text{C}$ and $^{36}\text{Ar}/\text{K}$ ratios for Earth and meteorites (Table 1) show considerable variation, however, and suggest that appreciable fractionation may have occurred in the $^{36}\text{Ar}/\text{K}$ ratio in the inner Solar System. Therefore this ratio cannot be assumed arbitrarily for Mars. The possibility of subsequent loss of ^{36}Ar from the Mars atmosphere^{16, 17} and the uncertainty in the relation between early catastrophic degassing of ^{36}Ar and continuous degassing of ^{40}Ar (see, for example, ref. 14) add additional variables to any interpretation of the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the Mars atmosphere.

It is also interesting to compare relative abundances of C, N and Ar recently determined in the atmosphere of Venus¹⁸ with those for Mars, meteorites and the Earth (Table 1). For the atmosphere of Venus the N/C ratio is similar to that of the whole Earth and meteorites, and the total amounts of N_2 and Ar in the Venus atmosphere are similar to total amounts in the Earth's atmosphere¹⁸. These observations suggest that large fractions of the C, N and ^{36}Ar on Venus may reside in its atmosphere. As for Mars, however, both the relative abundances of volatiles supplied to Venus and the fractions of original volatile elements which now reside in the atmosphere are unknown. If we assume that volatiles supplied to Venus had $^{36}\text{Ar}/\text{C}$ and $^{36}\text{Ar}/\text{N}$ ratios like the Earth's and that these ratios in the atmosphere of Venus reflect the whole planet inventory, we can calculate $^{40}\text{Ar}/^{36}\text{Ar}$ ratios for Venus of 800 and 1010, respectively, by the following formula and its equivalent for Ar/N :

$$[^{36}\text{Ar}/\text{C}]_{\text{Earth}} + [^{40}\text{Ar}/\text{C}]_{\text{Venus}} = [\text{total Ar}/\text{C}]_{\text{Venus}}$$

Similarly, if we assume volatiles supplied to Venus had $^{36}\text{Ar}/\text{C}$ and $^{36}\text{Ar}/\text{N}$ ratios like C1 chondrites, calculated $^{40}\text{Ar}/^{36}\text{Ar}$ ratios for Venus are 16,666 and 16,847 respectively. Calculated $^{40}\text{Ar}/^{36}\text{Ar}$ ratios of ~ 900 would suggest that either Venus has a $\text{K}/^{36}\text{Ar}$ ratio ~ 3 times that of the Earth or that radiogenic ^{40}Ar on Venus has been ~ 3 times more efficiently degassed into the atmosphere. The latter explanation seems to be consistent with the measured high surface temperature on Venus⁹ and the recent radar observations of apparent large volcanoes²⁰. Calculated $^{40}\text{Ar}/^{36}\text{Ar}$ ratios of $\sim 16 \times 10^3$, however, would imply either a $\text{K}/^{36}\text{Ar}$ ratio or a ^{40}Ar degassing fraction higher by a factor of ~ 57 on Venus compared to the Earth. It has been estimated that 7–90% of the Earth's total ^{40}Ar (depending on the K content adopted) has been degassed into the atmosphere^{2, 14, 21}. Therefore, to assume that volatiles on Venus have relative abundances like C1 chondrites would also require higher $\text{K}/^{36}\text{Ar}$ ratios than either the Earth or C1 chondrites (Table 1). The simpler explanation is that volatiles accreted by Venus had relative abundances similar to those on Earth, and that Venus has degassed a higher fraction of its ^{40}Ar . This explanation, however, depends on the assumption that the

$^{36}\text{Ar}/\text{N}/\text{C}$ ratios of the atmosphere of Venus are essentially identical to the whole planet inventory, which may not be true.

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Limits to palaeogravity since the late Precambrian

HYPOTHESES involving substantial changes in Earth radius over geological time can be tested by measuring palaeogravity at, or near, the Earth's surface. The measurements are obtained by comparing the pressure–temperature conditions in the upper mantle, disclosed by kimberlite nodules of known age, with the computed pressure–temperature conditions for that time¹. Recently published data for six groups of kimberlites, the earliest from the late Precambrian, are analysed here.

Temperature of formation of pyroxene–garnet bearing nodules can be determined by the diopside geothermometer^{2, 3}. Pressure can be obtained from the alumina content of enstatite⁴. An adjustment of -8×10^8 Pa has been applied to all pressures not corrected for presence of calcium and iron in the system^{5, 6}. An envelope drawn tightly around points on the pressure–temperature plot for each kimberlite suite has been used to measure the uncertainty in the pressure determinations (Fig. 1). Envelopes are elongated, their gradients at relatively low pressures similar to those expected for the palaeogeotherm. Gradients at relatively higher pressures, however, are sometimes steeper. Such 'inflected' geotherms probably indicate some kind of thermal instability in the mantle and so separate calculations have been made for the low and high pressure nodules in each kimberlite suite.

The pressure–temperature conditions in the upper mantle beneath stable continents at the present time have been computed by several workers from measured heat flow and plausible arrangements of heat producing elements and conductivities^{7–9}. The two geotherms plotted in Fig. 1 represent extreme conditions—average continental lithosphere with heat flux 1.0 hfu (ref. 7) and lithosphere beneath Montana with heat flux

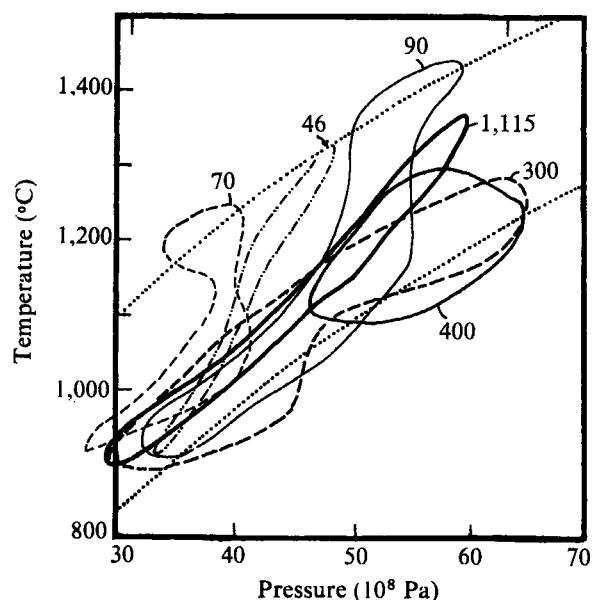


Fig. 1 Pressure-temperature conditions in the upper mantle deduced from ultramafic nodules in six kimberlite suites; ages in Myr BP. Dotted lines are limits to pressure and temperature beneath stable continents at present.

1.2 hfu (ref. 9). Heat production in the past was greater than now, however. For example, 1,100 Myr ago it was 1.2 times the present value¹⁰ and assuming thermal equilibrium the geothermal gradient would have been correspondingly steeper. For each kimberlite suite, therefore, a separate geotherm has been computed (not shown in Fig. 1) assuming a linear decline in heat production over 1,100 Myr.

A maximum limit to palaeogravity can be obtained by dividing the greatest pressure deduced from mineral chemistry ($P_m + p_m$) by the least pressure from the geotherm ($P_g - p_g$), both at the same arbitrarily chosen temperature T_m . Reversing the procedure gives a minimum limit. So

$$g_{\max} = [(P_m + p_m)/(P_g - p_g)]g_0$$

and

$$g_{\min} = [(P_m - p_m)/(P_g + p_g)]g_0$$

where P is mean pressure, p the uncertainty and g_0 the present value of gravity. The term involving the gradient of the geotherm formerly used¹ may be neglected. Limits derived from the above equations are given in Table 1. A reason for calculating limits rather than mean values for palaeogravity is

the difficulty of selecting a standard geotherm for all continental lithosphere. The difference between pressure-temperature plots for different kimberlites in southern Africa may lie partly in regional variations in the geotherm¹¹. Another reason is the possibility of systematic errors of 10^8 to 10^9 Pa in the pressure estimates derived from mineral chemistry. Nevertheless, the limits are probably more reliable than those derived by using a similar technique on upper mantle peridotites or metamorphic mineralogy¹ for which the geothermal regimes are even more uncertain.

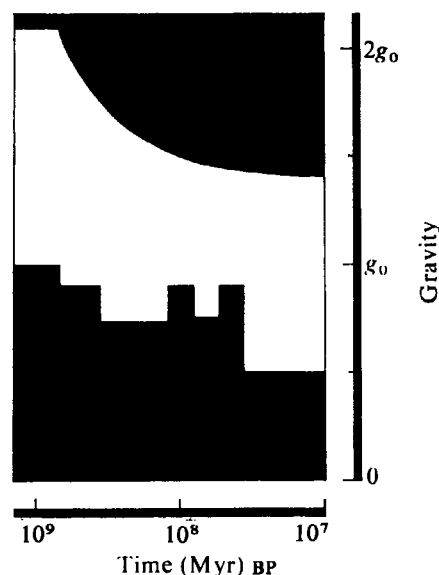


Fig. 2 Limits to palaeogravity over the period 100 Myr to 1,115 Myr. Lower limits come from the kimberlite data in Fig. 1. Upper limits are determined from metamorphic mineralogy (see text). Present gravity at the Earth's surface is g_0 .

The new results show that gravity since the late Precambrian could never have been much less than at present. The lower limit from the Premier Mine is particularly significant; it is fractionally greater than unity and thus lends no support to the most recent version of the contracting Earth hypothesis¹². This hypothesis requires virtually instantaneous core formation by phase change about 2,500 Myr ago, followed by a gradual contraction of Earth radius from 6,671 km (which implies $g = 0.9 g_0$) to the present value of 6,371 km.

The upper limits to palaeogravity obtained are of little use. A more stringent upper limit for the Phanerozoic ($g < 1.4 g_0$) from the non-occurrence of lawsonite in deep sedimentary

Table 1 Limits to palaeogravity derived from the envelopes in Fig. 1

Kimberlite suite	Ref.	Age (Myr)	P_m (10^8 Pa)	p_m (10^8 Pa)	P_g (10^8 Pa)	p_g (10^8 Pa)	$\frac{g_{\max}}{g_0}$	$\frac{g_{\min}}{g_0}$
Premier Mine	19	1,115	39.0	3.0	24.5	10.0	2.9	1.0
South Africa			55.5	1.5	38.0	15.0	2.5	1.0
Sloan II	20	400	56.7	7.3	42.0	12.0	2.1	0.9
USA			37.5	6.5	27.5	9.0	2.4	0.8
Udachnaya	21	300	56.5	6.5	43.0	12.0	2.0	0.9
USSR			39.7	3.7	31.0	11.0	2.2	0.9
Kimberley	3,22,23	90	52.0	3.0	57.0	14.0	1.3	0.7
South Africa			38.0	3.0	35.5	9.5	1.6	0.8
South West	24	70	37.0	3.0	48.0	10.0	1.1	0.6
Africa			36.5	0.5	32.0	8.0	1.5	0.9
Montana	25	46	47.0	1.0	59.0	13.5	1.1	0.6
USA								

The top line of data for each kimberlite suite relates to low pressures and temperatures and is probably the more reliable.

basins, has already been published¹. The limit rises to 2.0 g_0 at the end of the Precambrian when allowance is made for increased continental heat production. It compares with a Proterozoic maximum of 2.1 g_0 , using a compromise aluminium silicate triple point¹³ ($P_m = 4.8 \times 10^8$ Pa, $p_m = 1 \times 10^8$ Pa, $T_m = 575$ °C, $P_g = P_m$, $p_g = 2.0 \times 10^8$ Pa) and the equations given above. These upper limits flatly contradict rapid Earth expansion models^{14–16} unless mass increases with radius. Rapid expansion models require Earth radius to have been 4,500 km in the Carboniferous, 300 Myr ago, which gives $g = 2 g_0$. Slow Earth expansion^{17, 18} is still possible, however.

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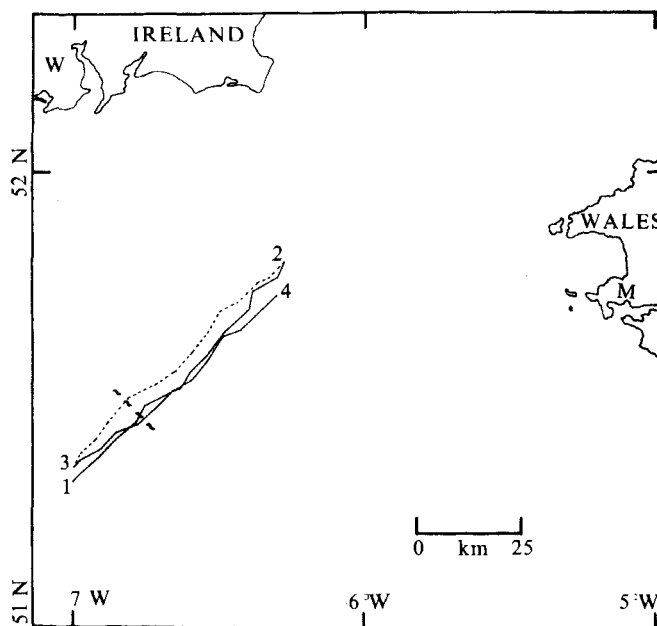


Fig. 1 Location of transects of Celtic Sea thermal front on 8 September 1976. ~ ~, Approximate position of main frontal boundary. Position 1, start of first transect; position 2, end of first transect and start of second; position 3, end of second transect and start of third; position 4, end of third transect. W, Waterford; M, Milford Haven.

the front, marked increases in productivity and chlorophyll were found to be associated with zones of increased lateral temperature gradient at the sea surface. In the third and final transect, however, this association between the physical and biological properties was masked by the development of sharp local increases in the magnitude of the biological characteristics. These local increases seemed to result from the greater degree of wind mixing experienced on this transect.

Continuous on-line analysis of the physical properties and the dissolved constituents along a transect approximately parallel to the tidal stream (Fig. 1) provided a detailed characterisation of the frontal structure. The essential physical features of the initial and final traverses are summarised in Figs 2 and 3 respectively, and the mean characteristics of the identified water types are listed in Table 1.

In the initial transect, waters exhibiting uniform characteristics typical of supra-thermocline waters in this region (type A) were terminated by a sharp thermal gradient (~ 1 °C mile⁻¹) and chemogradient (~ 75 μ g Si l⁻¹ mile⁻¹). Adjacent to the front a pronounced temperature minimum (the lowest recorded along the transect), with associated salinity minimum and nutrient maxima, indicated a narrow band of water with a significant upwelled component⁷. Between the main frontal features and the major body of cool water (C) lay a lens of warm water (B) bounded by minor but distinct thermal gradients, suggesting a zone of less vigorous mixing in the frontal circulation leading to localised surface heating.

A gradual deterioration in weather conditions accompanied subsequent transects. The essential hydrographic features observed in transect 1 were also evident in the second transect, although in general the boundaries were less well demarcated. Despite the

Table 1 Mean characteristics of the water types depicted in Figs 2 and 3

Water type	Salinity (%)	Temperature (°C)	Silicate concentration (μ g l ⁻¹)
A (Fig. 2)	35.10	17.50	26
B	34.88	15.70	165
C	34.90	15.25	185
A (Fig. 3)	35.05	17.65	20
D	35.15	16.70	125
E	34.85	15.80	200
F	34.90	15.20	220

Phytoplankton biology of a thermal front in the Celtic Sea

SEASONAL thermal fronts which occur in coastal waters are typified by sharp lateral gradients of temperature at the sea surface resulting from a surface outcropping of the thermocline. The frontal regions are areas of considerable physical and biological activity; in the case of the fronts in the Celtic and Irish Seas this increase in biological activity is reflected in marked increases of chlorophyll *a* at the sea surface¹. Considerable data on the spatial distribution of physical and chemical characteristics of the water and chlorophyll *a* concentrations in the vicinity of these and other fronts are now available^{2–5}. But, few observations have been made either on the temporal variability of these characteristics or on the more detailed distribution of phytoplankton productivity. During a survey of the Celtic Sea in September 1976 we made consecutive crossings of the frontal region in order to investigate the small-scale distributions of chlorophyll *a* and primary productivity and their inter-relationships with selected physical and chemical variables.

Temperature, salinity and concentrations of dissolved silicate and chlorophyll *a* were recorded continuously along the ship's track from water pumped through a port in the ship's hull at a depth of 2 m (ref. 2). Single observations of productivity were determined from phytoplankton samples also taken from this source. The productivity was estimated by the standard ¹⁴C uptake method⁶, samples being incubated for 3 h under a light intensity of 6 kl. The samples were cooled by pumping surface seawater through the incubator. During the first two transects of

increase in the wind-induced mixing, in the final transect the major thermal discontinuity, together with its boundaries of warm and upwelled water, was still prominent. Three additional distinct water types (D, E, F), demarcated by thermal, halo- and chemo- gradients, were evident in a direction away from the major discontinuity. Although the average temperature and salinity of water types E and F were similar to those of the corresponding water types B and C in the previous two transects, average silicate concentrations had increased significantly.

The chlorophyll *a* and productivity records associated with these transects are illustrated. Reference to Fig. 2 clearly demonstrates that the complementation of waters of inherently different properties induces fertility¹. Low chlorophyll *a* concentrations prevailed throughout the stratified waters on the warm side of the major thermal boundary and within the main water bodies of B and C, while chlorophyll peaks occurred at the thermal discontinuities. The fertilisation effect is further emphasised by the magnitude of the chlorophyll peaks at the discontinuities. Low chlorophyll *a* concentrations in the central body of the upwelled water increased towards the bounding thermal fronts. The increase in a direction towards the major temperature gradient was significantly less marked, however, reflecting the reduced degree of mixing between the contrasting water types adjacent to the major lateral density gradient. Maximum chlorophyll *a* concentrations occurred in the minor density gradient between water types B and C where a greater degree of lateral mixing was possible.

In the final transect chlorophyll *a* concentrations exhibited a marked spatial variability (Fig. 3). In contrast to the initial

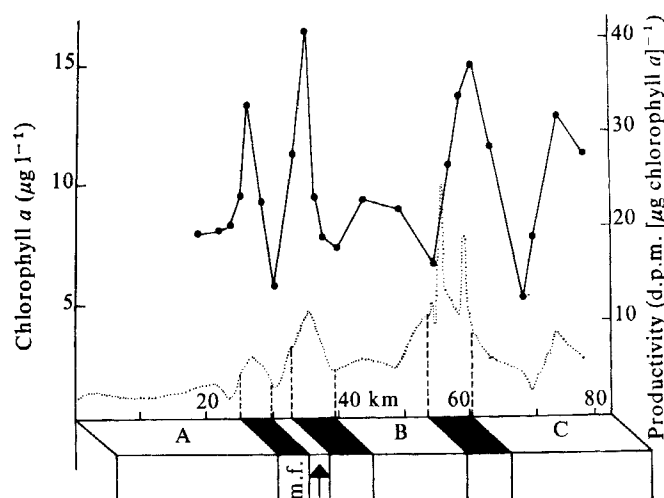


Fig. 2 The physical structure, chlorophyll *a* and productivity records observed during transect 1 across the front 0530 to 0930 8 September 1976. Abcissa denotes distance along the transect. Solid line represents productivity; dotted line represents chlorophyll concentration. Areas of sharp temperature gradient (■), the major front (m.f.), the upwelling zone (↑) and distinct water types are depicted.

transect, high concentrations not only occurred at the boundaries of the various water types, but also within them. Chlorophyll patches, approximately 0.75 miles across and containing up to $15 \mu\text{g l}^{-1}$, existed within water type E and persisted across the discontinuity into water type F. The chlorophyll patchiness coincided with noise on the temperature record indicating a causal relationship between patchiness and wind induced mixing within the bounds of the thermal discontinuities.

The productivity data, based on discrete samples, were less comprehensive. Nevertheless, the initial transect, which was preceded by a period of calm, depicted a distinct pattern of biological activity associated with the main physical features of the frontal system. A positive correlation between productivity and chlorophyll was clearly evident, with increases in productivity being particularly marked at the discontinuities. This relationship is associated with a later stage of development in frontal dynamics

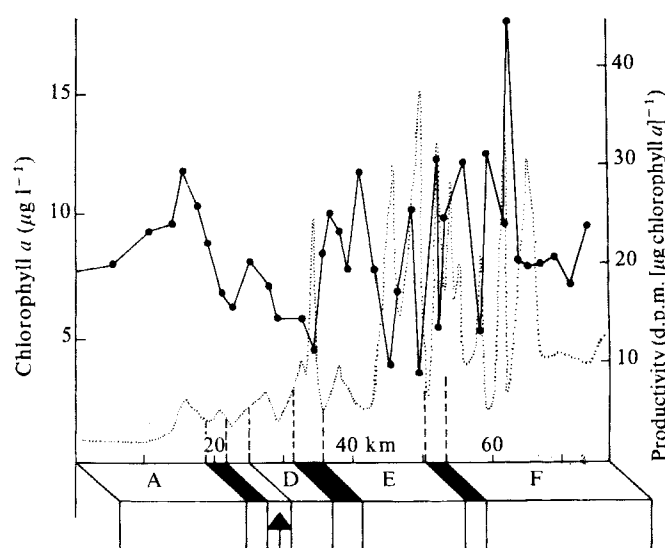


Fig. 3 The physical structure, chlorophyll *a* and productivity records observed during transect 3 across the front 1330 to 1720 8 September 1976. Abcissa denotes distance along the transect. Solid line represents productivity; dotted line represents chlorophyll concentration. Areas of sharp temperature gradient (■), the major front (m.f.), the upwelling zone (↑) and distinct water types are depicted.

during a period of stability. Sufficient time has elapsed for increases in productivity arising from the admixing of the contrasting water types to be reflected in an increase in phytoplankton stock, resulting in the observed positive correlation. For this condition to occur, it is clear that sufficient nutrients must remain in the mixed water in order to sustain a high level of production; also increases in phytoplankton must be in excess of losses from advection, diffusion and grazing. The corresponding early stage in the development of the dynamics will be represented by inverse correlations between these two characteristics: insufficient time will have elapsed for an increase in productivity resulting from admixing of water to be reflected in increases of the stock of phytoplankton.

In subsequent transects, wind induced mixing led to a rapidly evolving pattern of phytoplankton productivity and growth, resulting in a marked increase in the spatial variability of these parameters. The magnitude of the transient features of the biological characteristics was comparable to those observed earlier at the discontinuities. The spatial variability was especially evident in the cool waters of the frontal region in the final transect where an inverse relationship between productivity and chlorophyll was apparent. But, the increased mixing and spatial variability indicate that this relationship was not a reversion to an early stage in the sequential development of phytoplankton dynamics in a frontal region. Further support for this contention is available in the rate of evolution of the chlorophyll patches. Division time of phytoplankton in the sea averages 24 h^8 , whereas the chlorophyll transects showed at times a doubling of concentration within 6 h. The time scale of our observations therefore suggests that the development of the marked spatial variability in chlorophyll distribution was predominantly a consequence of physical aggregation and the outcropping of sub-surface concentrations of phytoplankton.

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A photoaffinity-labelled insect sex pheromone for the moth *Antheraea polyphemus*

SINCE the isolation of the first insect sex pheromone, bombykol, from the female silkworm *Bombyx mori*¹ and its full identification by synthesis as (10E, 12Z)-10,12-hexadecadienol^{2,3}, the number of characterised pheromones has increased dramatically⁴⁻⁷. The basic question of pheromone-receptor interactions remain unanswered, however. To devise a method which could be used to study such problems, it was necessary to modify the natural pheromone in such a way as to specifically label the binding site of its chemoreceptor cell. We chose to convert the acetate group, a common feature in Lepidoteran pheromones⁴⁻⁷, into a diazoacetate derivative. Information about the active site of the pheromone receptor cell can then be obtained by reacting it irreversibly with a carbene derived from the diazoacetate group. In this procedure, the modified pheromone is radioactively labelled, and since the carbene is generated photolytically, the process is called photoaffinity labelling^{8,9}. We have used electrophysiological techniques as a very sensitive and rapid bioassay method to check the activity of the modified pheromone. We recorded the responses from single receptor cells and evaluated the changes in receptor potential and nerve impulses activity accompanying exposure of the antennae to a stream of pheromone¹⁰⁻¹². We describe here experiments showing that the diazoacetyl derivative of the pheromone acetate (VII) meets the requirements of a photoaffinity label for the study of the receptor.

Fig. 1 Synthesis of the pheromone (6E, 11Z)-6, 11-hexadecadienyl acetate (VII) and its diazoacetate.

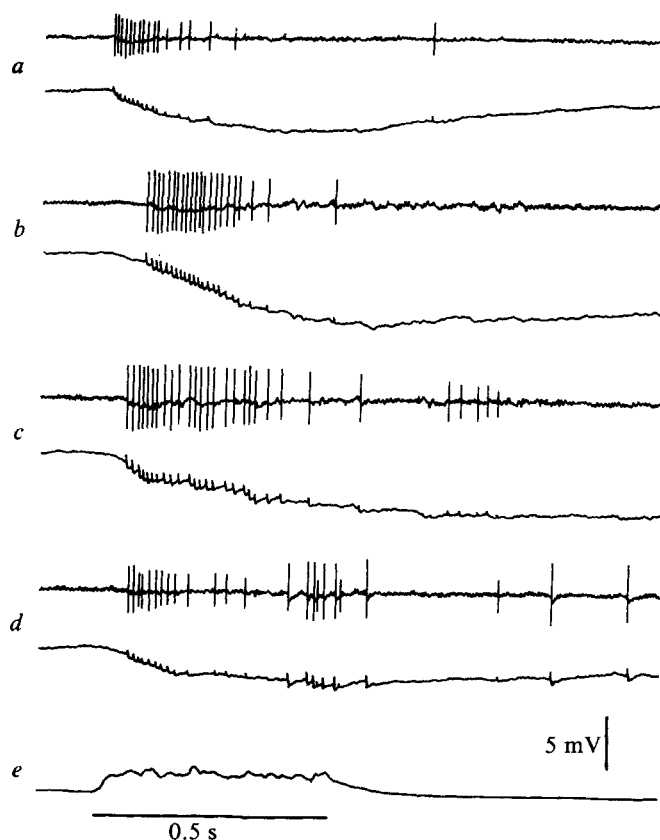
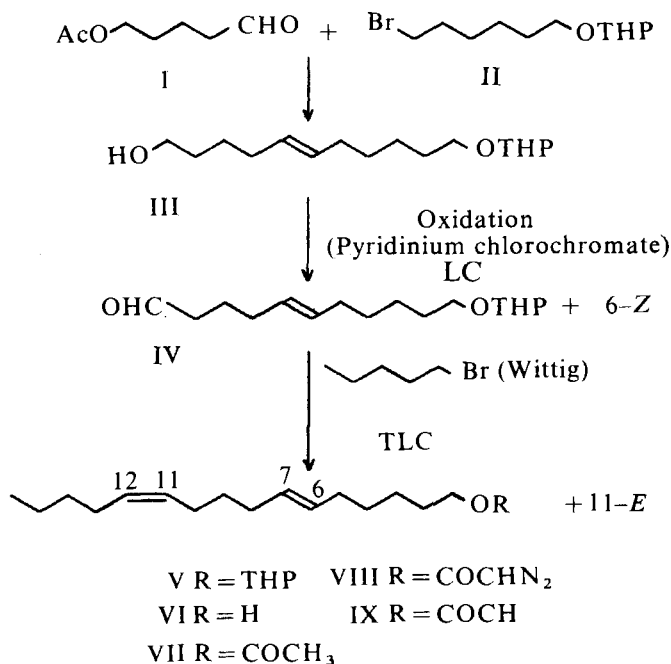


Fig. 2 Electrophysiological recordings from a single olfactory hair which is innervated by two receptor cells. *a*, One cell produces small nerve impulses and responds to (6E, 11Z)-6, 11-hexadecadienyl aldehyde 10^{-3} μg . *b*, The other cell fires large nerve impulses and is sensitive to (6E, 11Z)-6, 11-hexadecadienyl acetate 10^{-3} μg . *c*, The photoaffinity label (6E, 11Z)-6, 11-diazoacetate (DAC) at 10^{-3} μg excites only the 'acetate cell' (large impulses). The stimulus strength is given as the amount of each compound on a 1-cm² filter paper. *d*, The 'aldehyde cell' (small impulses) responds after irradiation of DAC with ultraviolet light (256 nm, 1 min on each side of the filter paper). The 'acetate cell' fires only very few impulses. Probably, most of the diazoacetate is converted into the aldehyde. *a-d*, Each response is given as a d.c. recording (lower traces) showing the receptor potential with superimposed nerve impulses, and as a.c. recording (upper trace) showing the enlarged nerve impulses. *e*, Response of a thermistor indicating the air-stream velocity at the antenna. The 5mV calibration refers to d.c. signals.

The sex attractants of the wild silkworm *Antheraea polyphemus* (Cramer), a saturniid distributed throughout North America, have been characterised as (6E, 11Z)-6, 11-hexadecadienyl acetate VII and its corresponding aldehyde by microchemical reactions, single cell recordings, and synthesis¹³. A 9:1 mixture of the acetate and aldehyde elicited strongest attraction to wild males in field tests.

The pheromones and derivatives were synthesised as outlined in Fig. 1. A Wittig reaction between acetoxy aldehyde (I) and tetrahydropyranyl (THP) derivative II of bromohexanol in ether -45°C for 80 min, followed by addition of ethanol, led to concomitant deacetylation to give a mixture of 6E-alcohol III and the 6Z isomer. The mixture was oxidised with pyridinium chlorochromate¹⁴ to the aldehydes which were separated by preparative liquid chromatography (LC) (Waters Prep LC500, 11% ether in hexane) using a silica gel column impregnated with 10% silver nitrate¹⁵. A second Wittig reaction between 6E aldehyde IV and bromopentane in ether at -45°C for 80 min followed by quenching with water, gave a 60:40 mixture of 11Z (V) and 11E THP ethers which were separated by preparative thin layer chromatography (TLC) on silver nitrate impregnated plates. Hydrolysis of the desired 6E, 11Z-THP ether (6% overall yield from acetate I) gave the alcohol VI which was

acetylated to pheromone VII. Reaction of alcohol VI with the *p*-toluenesulphonylhydrazide of glyoxylic acid chloride and triethylamine gave the diazoacetate VIII, with ultraviolet absorption bands (in hexane) of: 243 nm (10,000), 225 (8,900), 203 (12,700). Purity of final products and double bond isomers, except for the diazoacetate, was checked by gas chromatography.

Photolysis of the diazo group in diazo esters, for example VIII, affords the divalent carbene IX which is known to insert into nearby C-H, O-H, and N-H bonds^{8,9,16,17}. The diazoacetate VIII was relatively stable to sunlight; only 23% underwent photolysis when 60 µg in 3 ml hexane was exposed to sunlight for 60 min. But the same amount was totally photolysed after 10 s in a 254 nm Rayonet with 16 tubes.

It is now well-known that slight modifications in the structures of pheromones, for example, isomerism of double bonds or, especially, modification of terminal functions, lead to a drastic (10^{-1} to 10^{-3}) decrease in activity; also it is known that with certain insects the isomeric ratio of *Z/E* plays a critical part in pheromone reception^{18,19}. It was therefore gratifying to find that, when the 6*E*,11*Z*-diazoacetate VIII was submitted to electrophysiological tests (Fig. 2), it retained 10% of the activity of the natural pheromone VII and it elicited response of the acetate receptor cell dendrites but not of the aldehyde-sensitive cells. The 10% retention of activity has provided the incentive for further radiolabelled photoaffinity studies and detection of pheromone receptors.

Furthermore, electrophysiological experiments showed that neither *Antheraea roylei* nor *Bombyx mori* responded to the diazoacetate VIII (or acetate VII), thus indicating that the diazoacetate is not reacting with the *polyphemus* receptor cell in a random nonspecific manner. Interestingly, when a 1 cm² filter paper treated with 10^{-2} or 10^{-1} µg of the diazoacetate was irradiated for 1 min by an ultraviolet lamp, the aldehyde-sensitive cells responded but the acetate-sensitive cells did not.

It is possible that, in these conditions, the carbene undergoes a Wolff rearrangement to a ketene^{17,20} and that the ketene is further photolysed to the aldehyde²¹, the minor component¹³ of the natural pheromone.

Our results show that the diazoacetate equivalent of natural pheromones containing acetate groups, coupled with electrophysiological tests, could offer a promising lead to the study of pheromone receptors in insects.

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Infection of human pancreatic beta cell cultures with mumps virus

THE development of a diabetes-like syndrome in virus-infected animals, particularly in certain strains of mice infected with the M-variant of encephalomyocarditis (EMC) virus has recently been demonstrated^{1,2}. The possibility that viruses might also cause diabetes mellitus in humans, particularly in juveniles, has been suggested periodically since the turn of the century¹⁻⁶. The evidence, however, is largely circumstantial and comes from case reports showing a temporal relationship between the onset of certain viral infections, particularly mumps, and the subsequent development of diabetes. Although it has been known for some time that pancreatitis may be a complication of mumps⁷⁻¹⁰, specific involvement of the beta cells has never been demonstrated. Since it is not feasible to obtain pancreatic biopsies during the course of viral infections, we initiated experiments to determine if human pancreatic beta cells grown in culture were susceptible to mumps virus. We have used a double-label antibody technique, using fluorescein labelled anti-mumps antibody and rhodamine labelled anti-insulin antibody, and we show here for the first time that human beta cells can be infected with mumps virus.

Human pancreas was obtained at autopsy, usually within 12 h after death, from subjects aged 3 d to 46 yr, from the Division of Transplantation Surgery, Naval Medical Research Institute, Bethesda, and from the Department of Pathology, Children's Hospital, District of Columbia. Specimens were minced, washed in phosphate-buffered saline (PBS) and digested at 37 °C in PBS containing 0.1% collagenase (202 units mg⁻¹), 0.1% trypsin and 1.0% bovine albumin. Pilot experiments with 15 human and 50 non-human primate pancreas showed that this treatment was necessary to prevent gelatinous aggregation of the dispersed cells. The cells then were washed in PBS and resuspended in Ficoll-PBS (23.7% w/v) at a concentration of 2×10^7 cells ml⁻¹. A discontinuous Ficoll-PBS gradient was prepared with layers of 21.0%, 23.7% (containing cells) and 25.0%, and centrifuged at 350g at 4 °C for 20 min. The cells at the interface of the 23.7% and 25.0% layers were then decanted and cultured on glass coverslips in MPN165/C medium¹¹ with 10% heat-inactivated foetal bovine serum. Immunofluorescent staining of cells collected on a gradient with anti-insulin antibody demonstrated that approximately 1-5% of the cells were beta cells; a 10-fold enrichment over cells not passed through the gradient.

The ABC strain of mumps virus (from Dr Thomas Flanagan) was grown in African green monkey kidney cell line MA-134 (Microbiological Associates). Pancreatic cells that were in culture for 3-4 d were infected with mumps at a virus to total cells ratio of approximately 1.0. At appropriate intervals, coverslips were fixed for 10 min in acetone and stored at 4 °C until reacted with labelled antibodies.

Antiserum to bovine insulin prepared in guinea pigs (from Dr Peter F. Wright) was labelled with tetramethyl rhodamine isothiocyanate (TRITC; Baltimore Biological Laboratories)¹². The specificity of this reagent was determined by reaction of 6-µm cryostat-sectioned, acetone-fixed human pancreas. Only insulin-containing beta cells in the islets stained bright orange (Fig. 1a). The surrounding

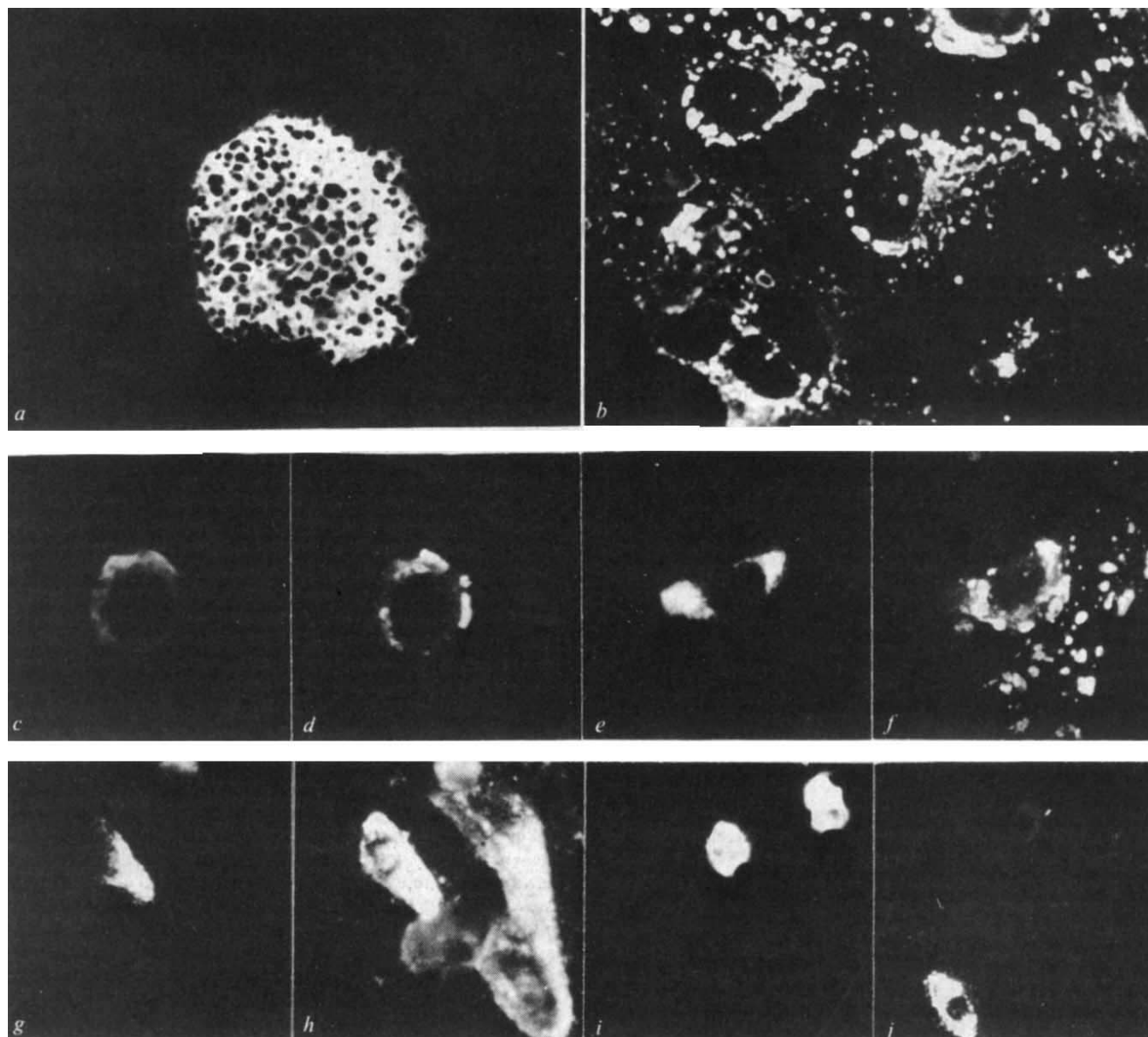


Fig. 1 *a*, Section of human pancreas stained with TRITC-labelled anti-insulin antibody. The photograph was taken with rhodamine filters. *b*, Mumps-infected MA-134 cells stained with FITC-labelled anti-mumps antibody (fluorescein filters). *c*, *e*, *g*, *i*, Human pancreatic cultures infected with mumps virus, stained with TRITC-labelled anti-insulin and FITC-labelled anti-mumps antibodies (rhodamine filters). *d*, *f*, *h*, *j*, Same cultures as above, but photographs taken with fluorescein filters. Magnifications: *b-h*, $\times 2,000$; *a*, *i*, *j*, $\times 800$.

acinar cells did not stain. Absorption of the labelled antibody with purified bovine insulin completely blocked staining (data not shown).

Antiserum to mumps virus, prepared in guinea pigs and labelled with fluorescein isothiocyanate (FITC), was purchased from Microbiological Associates. The specificity of the antiserum was demonstrated by reacting it with mumps-infected MA-134 cells. Mumps antigens stained brilliant apple-green, showed an irregular distribution in the cytoplasm, and seemed more globular than insulin antigens (Fig. 1*b*). Uninfected cells did not stain.

To identify mumps-infected beta cells, coverslips that had been prepared from infected and uninfected pancreatic cultures were stained first with TRITC-labelled anti-insulin antibody, washed and then stained with FITC-labelled anti-mumps antibody. When the order of staining was reversed the rhodamine intensity was slightly diminished, but specificity was not changed.

Coverslips were examined with a Standard Universal Zeiss Microscope illuminated by an HBO 200 mercury lamp. For detection of FITC-labelled anti-mumps antibody, KP490 and

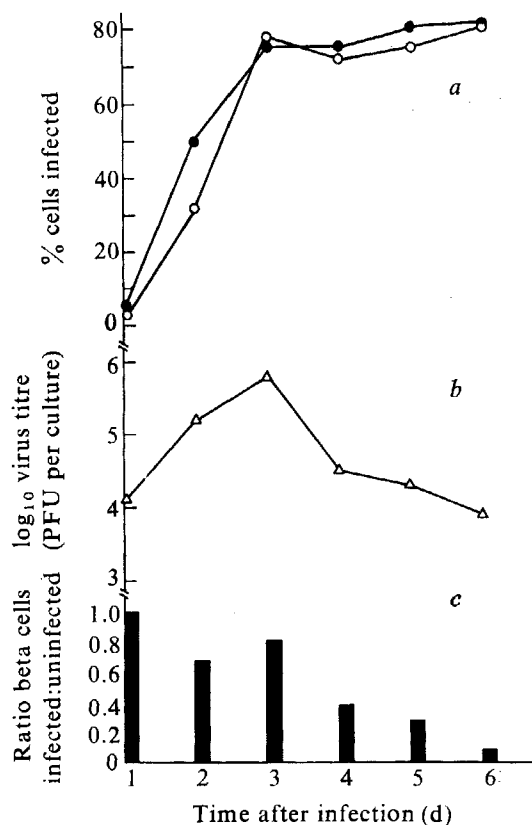
BG12 excitation filters and an LP528 barrier filter were used. For detection of TRITC-labelled anti-insulin antibody, a BP546/9 excitation filter and an LP590 barrier filter were used. Double-stained cells were identified by screening sections first with the rhodamine filters and then with the fluorescein filters.

Human pancreatic cultures were infected with mumps and at various times thereafter stained by the double-label antibody technique. Figure 1*c* and *d* shows a single cell containing both mumps antigen and insulin. When the rhodamine filters were used to examine the cell (Fig. 1*c*), a diffuse homogeneous orange colour, distributed throughout the cytoplasm, was observed. When the same cell (Fig. 1*d*) was examined using fluorescein filters, a brilliant apple-green colour was seen in the cytoplasm. Neither mumps nor insulin was detected in the nucleus. Similarly, the cell in Fig. 1*e* was identified as a beta cell by its orange appearance when viewed with rhodamine filters and as a mumps-infected cell by its green appearance (Fig. 1*f*) when viewed with fluorescein filters. The irregular distribution and globular appearance of the mumps antigen

is very similar to that seen in infected MA-134 cells (Fig. 1b). In addition, Fig. 1f shows mumps antigens in adjacent cells, but the lack of fluorescence in the corresponding positions in Fig. 1e, when rhodamine filters were used, indicates that these cells do not contain insulin. This is even more apparent by contrasting Fig. 1g and h, which show a single beta cell and a portion of a second beta cell (1g) infected with mumps (h) and several non-insulin-containing epithelioid cells also infected with mumps (h). Thus, by use of the double-label antibody technique it is possible to identify infected, insulin-containing, beta cells in a population consisting predominantly of infected non-beta cells. This technique also can be used to detect uninfected beta cells in a field of infected, non-insulin-containing cells. When the rhodamine filters were used, the two cells in Fig. 1i stained bright orange, indicating that they were beta cells; the lack of colour in corresponding positions in Fig. 1j, when the fluorescein filters were used, showed that these beta cells were not infected with mumps. The presence of green fluorescence in the lower left corner of Fig. 1j showed that this cell was infected with mumps, but the lack of orange fluorescence in the corresponding position in Fig. 1i indicated that it was not a beta cell.

To study in more detail the effect of mumps virus replication on pancreatic cell survival, cultures prepared from a single human pancreas were examined daily for 6 d. The percentage of beta cells and non-beta cells that became infected was evaluated by the double-label antibody technique. The data in Fig. 2 show that at 2 d after infection, 32% of the beta cells and 50% of the non-beta cells had become infected with mumps. At 3 d, approximately equal proportions (75%) of beta and non-beta cells

Fig. 2 Infection of human pancreatic cultures. a, Percentage of beta cells infected was determined by staining cultures with TRITC-labelled anti-insulin antibody and FITC-labelled anti-mumps antibody. ○, Beta cells; ●, non-beta cells. b, Viral titres were determined by assay on MA-134 cells and are expressed as plaque-forming units (PFU). c, The number of beta cells in infected as compared with uninfected cultures is expressed as a ratio.



were infected. Virus titres in the cultures increased from $10^{4.2}$ plaque-forming units (PFU) per culture at 24 h after infection to $10^{5.8}$ PFU at 72 h. The infectious titre declined over the next 3 d. The ratio of the number of beta cells in infected cultures to the number of beta cells in uninfected cultures declined from 1.0 at 24 h after infection to 0.1 at 6 d after infection. The highly lytic nature of the mumps infection points to beta cell death as the most likely explanation for this decrease rather than virus-induced degranulation. A similar decline in the number of non-insulin-containing cells in infected cultures also was observed.

In all, pancreatic cultures from seven subjects were examined for their susceptibility to mumps virus. Patients were of both sexes, and ranged in age from 3 d to 46 yr. The proportion of beta cells in these cultures, determined by staining with TRITC-labelled anti-insulin antibody, ranged from 1–5%. By the third day after infection, 60–90% of the beta cells in these cultures contained mumps antigens. Similarly, 60–95% of the non-insulin containing cells in the same cultures also contained mumps antigens.

By use of the double-label antibody technique, we have shown that human beta cells are susceptible to mumps virus. Moreover, by this technique we have shown that it is possible to identify infected beta cells in a population which contains predominantly (>95%) non-beta cells. In our experiments, beta cells from all seven human pancreas examined seemed to be susceptible to infection with the ABC strain of mumps. We have been able to show that beta cells from African green and rhesus monkeys also are susceptible to *in vitro* infection with mumps virus (data not shown). It must be emphasised, however, that these experiments by no means prove that mumps can produce diabetes. It remains to be determined whether the *in vitro* conditions may have altered the beta cells in such a way as to make them susceptible to a variety of viruses to which they would not have been susceptible *in vivo*. If future experiments show that beta cells grown in culture are restrictive to the replication of certain viruses, but allow the replication of other viruses, then this *in vitro* approach might serve as a useful screening technique for identifying viruses that have a tropism for human pancreatic beta cells and therefore have diabetogenic potential.

Because of the near universal prevalence of mumps, it is clear that if mumps virus is capable of infecting beta cells *in vivo* and producing diabetes, it must do so only in very special circumstances. That is, a particular variant of mumps virus must be involved and/or the individual who develops mumps must have an unusual, possibly genetically determined, susceptibility to the virus. Indirect evidence that a variant of mumps might be involved comes from epidemiologic studies which showed an increased incidence of juvenile diabetes following only certain mumps epidemics^{4–6}. It may be relevant that in mice, only the M-variant of the EMC virus group produces diabetes and the degree of diabetes varies with the passage history of the virus^{1,2}. Host defence mechanisms may also play an important part in determining whether an infected individual develops diabetes. Ordinarily, these host defences prevent the spread of the virus from the salivary glands to the pancreas. If, however, in unusual circumstances, mumps virus reaches the pancreas, the virus may infect and destroy beta cells. Whether such an infected individual develops diabetes may depend on the number of beta cells destroyed.

Finally, it is known that genetically determined host factors control the susceptibility of murine beta cells to EMC virus infection¹. In humans, the frequency of certain HLA antigens (for example, B8, BW15) is several times greater in insulin-dependent juvenile diabetics than the general population^{13,16}. By use of the double-label antibody technique, it might now be possible to determine if beta

cells from individuals with different HLA types show any difference in susceptibility to viral infections.

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Prostaglandins in urine of foetal lambs

ALTHOUGH prostaglandins of the E and F series have been measured in amniotic fluid from several species^{1–3} their principal site of synthesis remains uncertain. In man, for example, the decidua^{4,5} foetal membranes^{6,7} and myometrium⁸ have all been shown to synthesise prostaglandins *in vitro* and each may contribute to the prostaglandin composition of amniotic fluid. The foetal lung and kidneys secrete considerable volumes of fluid into the amniotic sac^{9,10} and hence may also contribute to the prostaglandin content of amniotic fluid. The levels of prostaglandins in foetal tracheal fluid have been shown to be much lower than those in amniotic fluid¹¹ and it was suggested that foetal urine is a more likely source of amniotic fluid prostaglandins. The foetal kidney is capable of synthesising prostaglandins¹² and there is a growing body of evidence that prostaglandins are intimately involved in renal function^{13–15}. We have, therefore, measured the concentrations of PGE, PGF and 13, 14-dihydro-15-keto-prostaglandin F (PGFM) in foetal urine during late pregnancy in sheep and have further determined the osmolality, Na⁺ and K⁺ concentrations of foetal urine and plasma. The concentrations of prostaglandins measured in foetal urine were similar to amniotic fluid levels but significantly greater than those previously reported in plasma and tracheal fluid suggesting that the kidney is a major source of amniotic fluid prostaglandins. The excretions of prostaglandins were related to the rate of urine flow; the excretions of PGF and PGFM were correlated with osmolar and solute-free water clearances and Na⁺ excretion, whereas excretion of PGE was related only to the clearance of solute-free water. Prostaglandins may thus contribute substantially to renal function and volume homeostasis in foetal life.

Four cross-bred sheep were used in this study. At laparotomy between 115 and 120 d of gestation catheters were implanted into a foetal femoral artery, tarsal vein and amniotic sac and also into the foetal urinary bladder using procedures described previously^{16,17}. Daily samples (2 ml) were taken from the femoral artery for measurements of P_{O_2} , P_{CO_2} , pH and haematocrit in whole blood and osmolality, Na⁺ and K⁺ concentrations in plasma. Foetal urine was collected for periods of 1–4 h by placing the free end of the bladder catheter 30 cm below the level of the foetus and allowing urine to drain by gravity. Plasma and urine samples were frozen immediately after collection and stored at –20 °C until analysis. P_{O_2} , P_{CO_2} and pH were measured using a Radiometer blood gas analyser. Osmolalities

Table 1 Prostaglandins in urine from four foetal lambs at 124–144 d gestation

	PGE	PGF	PGFM
Concentration (ng ml ⁻¹)	4.59±0.61 (18)	1.96±0.41* (23)	2.64±0.19† (23)
Excretion (ng min ⁻¹)	2.36±0.31 (18)	0.98±0.19* (23)	1.36±0.18† (23)

Values are means ± s.e.; figures in parenthesis are no. of samples.

* $P < 0.01$ compared to PGE.

† $P < 0.001$ compared to PGE.

were measured by freezing point depression (Advanced Osmometer) and Na⁺ and K⁺ concentrations were measured by atomic absorption spectrophotometry (Pye Unicam). Prostaglandins were measured by specific radioimmunoassays^{2,11}. Statistical differences were calculated using the Student *t* test and correlations were assessed by the method of least squares.

The mean concentrations and excretions of PGE, PGF and PGFM in urine sampled daily from foetuses between 120–144 d of gestation are shown in Table 1. The concentration of PGE was significantly higher than the concentration of PGF ($P < 0.001$) or PGFM ($P < 0.01$). There was no correlation between the concentrations of PGE and PGF but the concentrations of PGF and PGFM were significantly related ($r = +0.67$, $P < 0.001$, $n = 23$). The concentrations of PGE, PGF and PGFM in urine were significantly greater than those previously described in foetal plasma and tracheal fluid (Table 2).

The concentrations and excretions of prostaglandins were not related to the arterial blood gases, haematocrit, or the pH of either blood or urine. The excretion of PGE, but not PGF or PGFM, increased with gestational age ($r = +0.62$, $P < 0.01$, $n = 18$). The ratio of the mean concentrations of PGE and PGF was 2.34 (see Table 1), although the most commonly observed (modal) value was 1.25. The excretions of PGE, PGF and PGFM were significantly related to the rate of urine flow (Table 3). The excretions of PGF and PGFM correlated with osmolar and solute-free water clearances and also with Na⁺ excretion, whereas the excretion of PGE was related only to the clearance of solute-free water.

The relatively high concentrations of prostaglandins in foetal urine strongly suggest that the foetal kidney is an important source of prostaglandins in the foetus and probably a major source of the prostaglandins found in amniotic fluid. The excretion of PGE is similar to its concentration in amniotic fluid implying a high turnover in the amniotic fluid. Excretion of prostaglandins in urine closely reflects biosynthesis by the kidney¹⁸. Prostaglandins enter urine from the medulla at the ascending limb of the loop of Henle¹⁸ and, in the adult animal, undergo considerable intrarenal metabolism^{19,20}. Even assuming that minimal degradation of prostaglandins occurs within the

Table 2 Prostaglandins (ng ml⁻¹) in plasma urine, amniotic fluid, and tracheal fluid from foetal lambs

	PGE	PGF	PGFM
Urine	4.59±0.61 (18)	1.96±0.41 (23)	2.64±0.19 (23)
Plasma ³	0.34±0.028* (19)	0.177±0.013* (19)	—
Amniotic fluid ¹¹	2.54±0.41† (21)	3.78±0.97 (21)	2.88±0.26 (21)
Tracheal fluid ¹¹	0.96±0.19* (21)	0.33±0.07* (21)	0.77±0.07* (21)

Samples of plasma, urine, amniotic fluid and tracheal fluid were obtained from different foetuses at various times during the 1975–76 sheep season in Oxford. Values are means ± s.e.m. with no. of observations in parentheses.

* $P < 0.001$ compared to prostaglandins in urine.

† $P < 0.01$ compared to prostaglandins in urine.

Table 3 Correlation of prostaglandin excretions (ng min⁻¹) with urine flow, osmolality, osmolar clearance, solute-free water clearance and sodium excretion

	<i>U</i> (ml min ⁻¹)	<i>U</i> _{osm} (mOsm/kg H ₂ O)	<i>C</i> _{osm} (ml min ⁻¹)	<i>C</i> _{H₂O} (ml min ⁻¹)	Na excretion (μEq min ⁻¹)
PGE excretion (<i>n</i> = 18)	+0.46*	NS	NS	+0.58*	NS
PGF excretion (<i>n</i> = 21)	+0.53*	NS	+0.45*	+0.65†	+0.49*
PGFM excretion (<i>n</i> = 21)	+0.86†	NS	+0.74†	+0.80†	+0.44*

Values are Pearson correlation coefficients: *U*, urine flow; *U*_{osm}, urine osmolality; *C*_{osm}, osmolar clearance; *C*_{H₂O}, solute-free water clearance. NS, not significant.

**P* < 0.05.

†*P* < 0.001.

‡*P* < 0.01.

foetal kidney the excretion rates in the present study are high compared with the conscious adult rabbit²¹ and anaesthetised dog¹⁸. The foetal lamb kidney can metabolise prostaglandins¹², however, and relatively high levels of PGFM were found in foetal urine. Hence it is possible that the foetal kidney has a greater capacity for prostaglandin biosynthesis than that represented by the direct excretions of PGE and PGF.

The high concentrations of prostaglandins found in urine have important implications for several aspects of renal function in foetal life. Renal prostaglandins are known to modulate renin release, salt and water homeostasis and to potentiate adrenergic nerve activity (for recent reviews see refs 22, 23). It is possible that renal production of PGE may be partly responsible for the hypotonicity of foetal urine and poor concentrating ability of the kidney since PGE is known to antagonise the actions of vasopressin on the collecting duct²⁴ and to increase independently solute-free water formation by an action on the proximal tubule²⁵. This hypothesis is supported by the positive correlation found in the present study between the excretion of PGE and solute-free water clearance in foetal urine. Since prostaglandins may modulate the secretion of renin from the kidney²⁶ they may be responsible for the relatively high renin activity of foetal plasma²⁷. The correlation between the excretion of PGF and osmolar and Na⁺ excretion is consistent with the renin inhibiting actions of PGF_{2α} (ref. 28).

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Physical and physiological properties of dry lung surfactant

THE phospholipids of pulmonary surfactant¹ cover the respiratory epithelium and because of their unique properties of lowering surface tension, spreading rapidly and sustaining high surface pressures, allow the lungs to expand easily and prevent their collapse. Deficiency of lung surfactant is the major cause of respiratory distress syndrome in premature babies and also contributes to the respiratory problems of paraquat poisoning and cardiac bypass surgery. It has generally been assumed that surfactant, whether in the lamellar bodies or alveoli, is in an aqueous environment and must, therefore, be hydrated. We show here, however, that fully hydrated surfactant is apparently inactive and that surfactant can only be effective as a surface monolayer if the source of the material is 'dry'.

The critical micelle concentration (CMC) of the principal lung surfactant phospholipid, L-α-dipalmitoyl phosphatidylcholine, in water has the extremely low value of $4.6 \pm 0.5 \times 10^{-10}$ M (ref. 2). In consequence the overwhelmingly preferred domain for a single phospholipid molecule in water is in association with other phospholipid molecules. The resulting aggregates, technically referred to as smectic mesophases or liposomes, come to occupy a minimum space in the hostile environment of water. The reason for the low CMC of the molecule is the large and incompatible-in-water hydrocarbon moiety.

A liposome infrastructure³ is characterised by alternating bimolecular sheets of lipid intercalated by aqueous spaces, whereby each and every lipid sheet forms a 'closed' membrane system and where all the hydrocarbon chains are screened from water by the polar head-group region (Fig. 1a). It seems unlikely that this material, in a fully hydrated state, could facilitate a rapid extension of an air/fluid interface by donating surface-active molecules, for example, as occurs during the first inspiratory breath of an infant. Nevertheless there has been a tacit assumption that the lamellar bodies of alveolar type 2 cells are packages of lung surfactant, smectic mesophase in character, in equilibrium with cell water.

We suggest that the lamellar bodies are packages of phospholipids in equilibrium with restricted amounts of water—less than about 15 water molecules per phospholipid, at which proportion the bimolecular sheets of phospholipid are not closed but 'open' and the structure has more of the properties of a water-in-oil system than an oil-in-water system. On release from a cell close to an advancing air/fluid interface the phospholipids would rapidly and easily donate molecules to the interface, since these molecules do not have to surmount the unfavourable hydration barrier (Fig. 1b).

The surfactant used in our experiments was prepared by washing out sheep's lungs with saline, removing the cellular debris by centrifugation, concentrating the fluid by lyophilisation and extracting the complete lipid content by the Folch procedure⁴. The chloroform layer was evaporated to dryness under nitrogen leaving a dry, waxy material, the surfactant. This has been analysed and found to contain all the generally accepted surfactant lipids in normal proportions (J. Harwood, personal communication). In the experiments using wetted surfactant, liposomes were formed by sonicating the dry surfactant in saline. Surface tension measurements were made using a roughened platinum dipping plate (2 cm wide) suspended from a force transducer feeding into a recorder. The liquid was contained in a clean, Teflon trough.

When a particle of dry surfactant was placed on the clean surface of physiological saline the surface tension always fell rapidly to the equilibrium tension of $\sim 24 \text{ dyn cm}^{-1}$ (Fig. 2a). If a similar amount of wet surfactant was added to the surface there was either no effect on surface tension or else it fell very slowly to some variable, intermediate value (Fig. 2b).

In other experiments we rapidly removed as much of a surface monolayer as possible by aspiration with a fine tipped sucker (Fig. 2 at each arrow). When the surface layer had been formed from a particle of dry surfactant the surface tension rose with aspiration and then quickly fell again, within 20 s, showing that more molecules were being donated to the surface. This could be repeated many times, but only as long as there was a particle of surfactant at the surface to act as a molecular reservoir (Fig. 2a). Once the particle was removed further recruitment of molecules to the surface became very slow. When wet surfactant was used and the surface monolayer was aspirated, the surface tension rose and fell again very slowly because few molecules were being recruited to the surface (Fig. 2b).

The effect of dry surfactant on lung expansion was investigated by placing a small particle into the fluid-filled trachea of dead 27-d foetal rabbits (term is 31 d, and the lungs contain little endogenous surfactant at this age). Twenty-one foetuses from six litters were used; 22 alternate foetuses were used as untreated controls. A tracheostomy tube was connected to a closed circuit apparatus constructed to inflate and deflate the lungs and to plot pressure volume curves. Three cycles were performed on each lung at 37°C . Twenty-nine foetuses were subjected to pressures up to $35 \text{ cm H}_2\text{O}$ and 14 foetuses to pressures up to $30 \text{ cm H}_2\text{O}$. There was no difference in body weights and wet lung weights between the treated and control

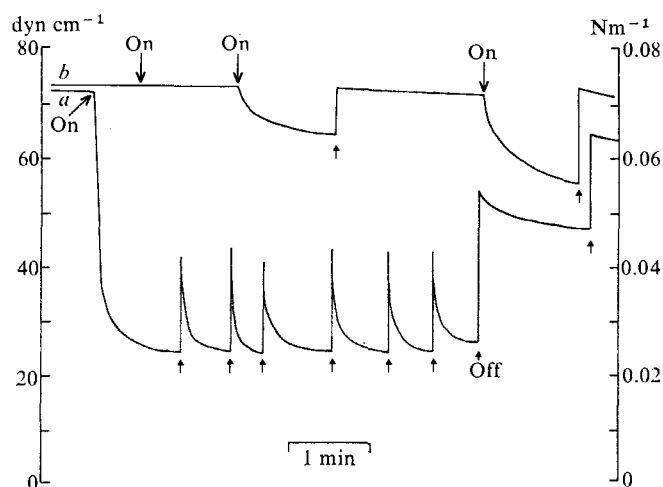


Fig. 2 The surface tension lowering properties of a, dry surfactant and b, wet surfactant placed on saline. In both instances surfactant was placed on the surface at 'On'. The dry particle was removed from the surface at 'Off'. Upwards-pointing arrows indicate when the surface monolayer was partly removed by aspiration.

foetuses. All the 21 treated lungs expanded at opening pressures below $30 \text{ cm H}_2\text{O}$. Only 6 of the 22 untreated lungs expanded, all with opening pressures above $30 \text{ cm H}_2\text{O}$. The mean volume retained at atmospheric pressure was $0.41 \pm 0.75 \text{ ml}$ (s.e.m.) for the treated lungs and $0.038 \pm 0.012 \text{ ml}$ for the untreated. These differences are statistically significant.

An intact surfactant monolayer is vital to the healthy functioning of the lungs. It allows the lungs to expand with a minimal expenditure of energy and prevents atelectasis in expiration. To be effective a surfactant must maintain the monolayer from the moment of birth. If the surfactant in the lungs is fully hydrated it will not form a surface active layer easily or quickly and will not spread fast enough to maintain an intact surface film. From these experiments we have shown that the active form of surfactant is the 'dry' state. We postulate that the lamellar bodies in the alveolar type 2 cells, which are known to be surfactant stored ready for release into the alveoli, are packages of surfactant in a 'dry' state.

There have been many attempts to treat respiratory distress syndrome in the newborn with surfactant substances as a mist nebulised with water. Not surprisingly this has proved uniformly ineffective. Our results suggest that it may be possible to correct surfactant deficient states with 'dry' surfactant.

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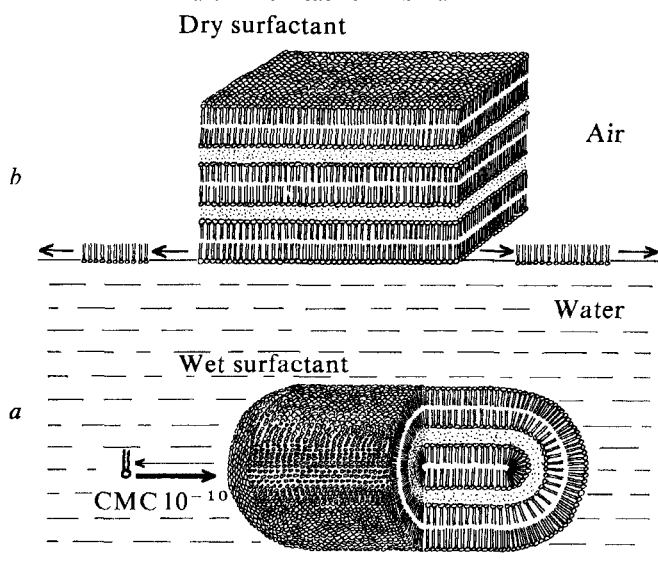
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Fig. 1 The molecular configuration of dry and wet surfactant. b, The dry surfactant has open ended layers from which molecules can spread freely to an air/water interface. a, In water the surfactant aggregates as a smectic mesophase and few molecules are free to reach the interface.



Vulnerability of methylcholanthrene-induced tumours to immunity aroused by syngeneic foetal cells

It is a well-observed fact that the growth of malignant tumours in experimental animals and in man is often accompanied by the renewed formation of embryonic or foetal substances the synthesis of which would normally have been switched off much earlier in life. This manifestation of anaplasia has often been reviewed¹⁻³. Among such foetal substances are antigens capable of arousing a cell-mediated immunity (CMI) directed both against embryonic cells and also against tumours. This makes it possible to perform experiments which would normally have been prohibited by the fact that the tumours induced by chemical oncogens are almost always antigenically *sui generis*. We present evidence that protection against tumours is secured by immunity to embryo-specific antigens aroused by normal pregnancy or by deliberate inoculation of embryonic cells. This might explain the known association between childbearing and the diminished risk of breast cancer.

A team at this institute had already shown² that the inoculation into CBA mice of irradiated cells isolated from 9-11-d-old CBA embryos 14 d before the subcutaneous injection of 50 µg of 3-methylcholanthrene (MCA) retards the onset and diminishes the final total frequency of tumours. They showed at the same time that when the inoculation of foetal cells was postponed until shortly before the first tumours were expected to arise (about 90 d), the MCA-induced tumours arose more quickly and in more mice. Further investigation (Fig. 1) confirmed the protective power of a foetal inoculum given 14 d before the administration of MCA, and showed again that an inoculum delayed until 14 d after MCA promoted the growth of tumours. The injection of a miscellany of irradiated adult CBA cells 14 d before MCA (under the conditions in which foetal cells are inhibitory) was without effect. In this experiment, however, the injection of adult cells 14 d after MCA caused some tumour promotion. This phenomenon, and the necessity for irradiation of the foetal inoculum if foetal cells are to be immunogenic, are still unexplained.

The balance between tumour promotion and tumour inhibition was now investigated in an experiment in which the ostensibly protective inoculum of foetal cells was administered 7 or 14 d before, or 7 or 14 d after the injection of 50 µg MCA.

The experiment was so arranged that the operation least easy to make uniform was carried out with a single homogeneous

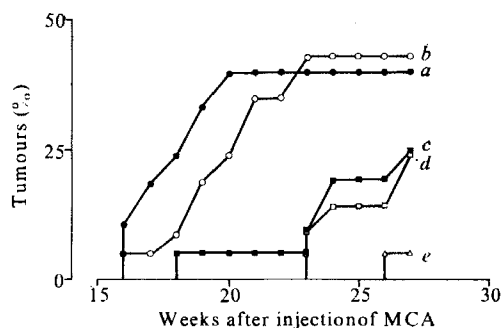


Fig. 1 Cumulative curves of tumour incidence in male CBA mice which, in addition to receiving 50 µg 3-methylcholanthrene (MCA), received inoculation of 1×10^6 foetal or adult cells at various intervals before (—) or after (+): A, foetal cells 14 d after MCA (+14); B, adult cells (+14); C, MCA alone; D, adult cells (—14); E, foetal cells 14 d before MCA (—14). Note protective power of foetal tissues given before (E) but not after (A) MCA. Note also that curves C and D are virtually indistinguishable, showing that adult cells are ineffective under the conditions in which foetal cells confer protection. Adult cells injected 14 d after MCA (B) secure some degree of promotion.

batch of irradiated CBA foetal cells on a single occasion: each of 125 female CBA mice of the same provenance received 1×10^6 irradiated (2,000R) foetal cells belonging to a single batch isolated on the same occasion from 10-11-d-old CBA embryos. The injections of MCA (0.1 ml per mouse 5% MCA in olive oil) were so arranged that each of five groups of 25 mice received their injections of MCA 7 or 14 d before or after, or at the same time as, the injection of foetal cells, at the times indicated in Fig. 2. For logistic reasons, mice receiving MCA alone could not be included, but internal comparisons between the groups show very clearly that inoculation of embryonic cells 7 or 14 d after or at the same time as the injection of MCA was much less effective than the inoculation of foetal cells 7 or 14 d before MCA.

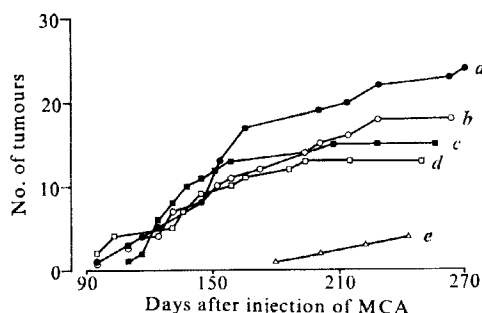


Fig. 2 Cumulative curves of tumour incidence in female CBA mice which, in addition to receiving 50 µg MCA received inocula of 1×10^6 foetal cells either on the same occasion (0) or at 7 or 14 d before (—) or after (+): A, foetal cells +14; B, foetal cell injection +7; C, foetal cells and MCA simultaneous; D, cell injection —7; E, foetal cells —14. Note relative effectiveness of foetal tissue given before MCA (E, D), compared with the effect of a cellular inoculation at the same time as (C), or after MCA (A, B).

The failure of experiments of therapeutic rather than prophylactic design, and the fine balance between discouragement and promotion of the growth of tumours may perhaps explain the disappointing clinical results of specific tumour therapy. They may also help to account for some unexpected characteristics revealed in the large-scale multi-centre epidemiological studies of the Harvard School of Public Health^{4,5} on the influence of parity on susceptibility to mammary cancer in human beings: the risk of breast cancer decreases with increasing parity, but the principal determining factor is the age of the mother at the birth of her first child; the relatively protective effect of a completed pregnancy before the age of 20 may last for 50 years. The explanation of this phenomenon may be endocrinological, but there is strong evidence that it is immunological: Brawn⁶, Hellstrom⁷ and Simpson and Gautam (personal communication) have shown that pregnancy in mice excites the formation of lymphocytotoxic cells directed against foetus-specific and tumour antigens; Moon⁸, moreover, has shown that rats which have borne litters show an enhanced resistance to the mammary tumours induced by the oral administration of dimethylbenzanthracene. The Harvard collaborative study made it clear that women who bore their first children at the age of 33 or later were more susceptible than childless women to mammary tumours. So far from being inconsistent with an immunological interpretation, this finding is what might have been anticipated from our own experimental results. The effect of an early pregnancy is in a sense simulated by the results of experiments in which immunity is aroused before the application of the oncogenic stimulus; the lessened effectiveness, even increased hazard associated with late pregnancy is simulated by the enhancing effect on tumour growth consistently found when the ostensibly protective inoculation is deferred until after the application of the oncogenic stimulus. The underlying assumption in both situations is that the transformation from normal

to malignant growth occurs very shortly after inoculation of MCA and very early in reproductive life.

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H-2 Antigens on a murine lymphoma are associated with additional proteins

THERE have been reports indicating that the normal phenotypic expression of histocompatibility antigens is altered on tumour cells¹, and a disappearance or diminution of H-2 expression on murine tumour cells has been demonstrated^{2,3}. The nature of these changes has so far remained unclear. It has also been shown that H-2D and H-2K antigens are important in T-cell mediated lysis of virus infected or transformed syngeneic cells^{4,5}. One explanation for these phenomena proposes that H-2 antigens are modified by association with additional cell-surface molecules to form a neoantigen or altered self⁶. We report here immunochemical evidence for alteration of H-2 antigens on tumour cells by association with additional proteins.

An oestrogen-induced lymphoma (6C3HED) (obtained from Dr M. Prager, Dallas, Texas) was maintained in syngeneic C3H mice by weekly intraperitoneal transfer of approximately 10⁶ tumour cells. We have observed that H-2.23, the private K locus antigen of the H-2^k haplotype, is undetectable on intact 6C3HED lymphoma cells by either cytotoxicity or absorption assays. This is in agreement with the results of Garrido *et al.*⁷. We have also observed, however, that a cell lysate produced by repeatedly freezing and thawing 6C3HED cells completely and specifically blocked the cytotoxic action of anti-H-2.23 on syngeneic normal cells. These data suggest that H-2.23 may be present on 6C3HED cells, but modified in such a way as to be undetectable on the intact tumour cell.

Normal C3H splenocytes and tumour cells were radiolabelled, extracted with 0.5% NP-40, and reacted with either anti-H-2.23 or normal mouse serum. The resulting antigen-antibody complexes were precipitated with Protein A-bearing *Staphylococcus aureus* and analysed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE).

There was a marked difference in the reactivity of anti-H-2.23 with normal C3H cells and with 6C3HED tumour cells (Fig. 1). Proteins with mobilities corresponding to molecular weights of 45,000 and 12,000 characteristic of detergent-solubilised H-2 and β_2 -microglobulins (β_2 - μ), respectively, were present in both electropherograms. Additional major peaks with mobilities indicative of molecular weights of approximately 70,000 and 55,000 were evident in the 6C3HED cell extract. To establish further that the protein peaks at 45,000 and 12,000 were H-2 and β_2 - μ and to determine whether H-2 antigens on the tumour cells are associated with β_2 - μ , additional experiments were carried out using a rabbit anti-rat β_2 - μ . This antiserum cross reacts with mouse β_2 - μ (ref. 8) and was prepared against homogeneous rat urinary β_2 - μ , which had been shown to give a unique amino acid sequence (M. Poulik, D. Schinnick and O. Smithies, unpublished): the specificity of this antiserum is known⁸. SDS-PAGE analyses of antigens isolated from biosynthetically labelled C3H splenocytes or 6C3HED tumour cells with rabbit anti-rat β_2 - μ are shown in Fig. 2. When tested with normal splenocytes, the anti- β_2 - μ produced peaks characteristic of β_2 - μ and detergent-solubilised H-2. When reacted with tumour cells, this antiserum also

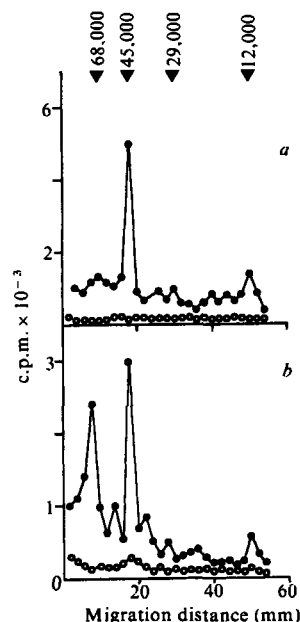


Fig. 1 SDS-polyacrylamide gel electrophoresis of murine lymphoid cell antigens isolated with anti-H-2.23 antiserum (K603). Cells were teased from whole C3H spleens or obtained from the ascites fluid of tumour-bearing mice, washed, counted, and adjusted to a concentration of 5×10^6 viable cells per ml in RPMI 1640 containing $100 \mu\text{Ci ml}^{-1}$ ^3H -leucine and incubated for 5 h at 37°C in a 5% CO_2 -95% air mixture. The cells were then collected, washed, and extracted in 0.5% NP-40 (v/v) in Tris-buffered saline (0.05 M, pH 8.0) for 30 min at 4°C . The extract was centrifuged at $45,000g$ for 15 min and the pellet discarded. Aliquots ($100 \mu\text{l}$) of the supernatant were incubated with $20 \mu\text{l}$ of antiserum for 1 h at 37°C , then overnight at 4°C . Indirect immunoprecipitation with $250 \mu\text{l}$ of 10% v/v heat-killed formalin-fixed *Staphylococcus aureus* (Staph A) (Cowan I Strain) and SDS-PAGE on 12.5% gels was carried out as described by Kessler¹⁶. a, N.C3H splenocytes; b, 6C3HED lymphoma cells. ●, Anti-H-2.23; ○, normal mouse serum. Molecular weight standards were bovine serum albumin, ovalbumin, carbonic anhydrase and cytochrome c.

produced the higher molecular weight peaks which were obtained with anti-H-2.23 as well as the H-2 and β_2 - μ peaks. Similar peaks were obtained when anti-H-2.23 and anti- β_2 - μ were reacted with NP-40 eluates of tumour cells which had been radioiodinated with lactoperoxidase. This indicates that the antigens detected were present on the cell surface.

To eliminate the possibility that antibodies reactive with antigens other than β_2 - μ were responsible for the precipitation of additional proteins, the rabbit anti-rat β_2 - μ was prereacted with highly purified rat β_2 - μ or bovine serum albumin (BSA) and then added to a radiolabelled extract of 6C3HED cells. The result of SDS-PAGE analysis of the precipitate is shown in Fig. 2b. The fact that preincubation of the antiserum with purified β_2 - μ reduced all peaks to background level, whereas prereaction with BSA had no effect, strongly suggests that the reactivity of this antiserum with the 6C3HED can be attributed to anti- β_2 - μ antibodies.

Although the reactivities of both anti-H-2 and anti- β_2 - μ antisera with normal and tumour cell extracts were very similar, it was not obvious whether or not they were reactive with the same antigenic structures. Immunoabsorbents (IADS) were therefore prepared by covalent attachment of crude Ig fraction of NRS or rabbit anti-rat β_2 - μ to CNBr-activated Sepharose 4B (ref. 9) and were incubated overnight with radiolabelled extracts of 6C3HED tumour cells. The IADS was then removed by centrifugation and the supernatants immunoprecipitated with anti-H-2.23 or rabbit anti-rat β_2 - μ . SDS-PAGE analyses of these immunoprecipitates are shown in Fig. 3. Precipitation of the H-2, β_2 - μ , and the higher molecular weight peaks by anti-H-2.23 or anti- β_2 - μ was unaffected by previous reaction of the tumour cell extract with the NRS immunoabsorbent. Previous reaction of the tumour cell

extract with the anti- $\beta_2\text{-}\mu$ IADS, however, resulted in removal of all proteins reactive with either anti-H-2.23 or anti- $\beta_2\text{-}\mu$.

There are several possible explanations for the coprecipitation of H-2, $\beta_2\text{-}\mu$, and the higher molecular weight proteins from tumour cell extracts following reaction of both anti- $\beta_2\text{-}\mu$ and anti-H-2 with the lymphoma cells. First, both K603 (anti-H-2.23) and the anti- $\beta_2\text{-}\mu$ sera could be contaminated with additional antibody. We have not established definitely that this is not the case with the anti-H-2.23 antisera, however, since the rabbit anti-rat $\beta_2\text{-}\mu$ was prepared in rabbits against highly purified rat urinary $\beta_2\text{-}\mu$, it seems unlikely that this antiserum was contaminated with additional antibodies reactive with material present only on mouse tumour cells. Second, the apparent association might be an artefact of the extraction procedure, but the proteins are probably not part of a large lipid-detergent micelle since H-2K, H-2D and Ia antigens present in similar extracts of normal C3H splenocytes are easily separated. Complex formation due to disulphide interchange during extraction is also unlikely since identical electropherograms were obtained when the extraction was carried out in the presence of 10 mM iodoacetamide. The appearance of additional proteins in the immunoprecipitate from the tumour cell extracts is not caused by nonspecific binding of proteins to the antigen-antibody complex, since the addition of unlabelled $\beta_2\text{-}\mu$ to the reaction mixture prevented the precipitation of any radiolabelled material. Clearly, then, $\beta_2\text{-}\mu$ protein complexes not present in extracts of normal cells are found in extracts of the 6C3HED tumour cells.

Two explanations exist to account for the relationship between the reactivities of the rabbit anti-rat $\beta_2\text{-}\mu$ and the anti-H-2.23. First, the anti-H-2 antiserum could contain additional antibodies which are reactive with material present on tumour cells but absent from normal cells. These additional antigens, as well as H-2, must be associated with $\beta_2\text{-}\mu$ since prereaction of the 6C3HED cell extracts with the anti- $\beta_2\text{-}\mu$ adsorbent removed all material reactive with anti-H-2. To date, $\beta_2\text{-}\mu$ has been shown to be associated only with H-2 (ref. 10), TLA (ref. 11), and Qa-2 (ref. 12) on the surface of normal cells. A second possibility is that a complex composed of at least one $\beta_2\text{-}\mu$, one H-2 heavy chain and one or two higher molecular weight proteins is formed on the tumour cell. In detergent extracts, this complex precipitates with anti-H-2.23 and anti- $\beta_2\text{-}\mu$ as a single entity. The complex is reduced to its individual protein components when the precipitates are prepared for SDS-PAGE.

At present, we cannot distinguish unequivocally between these two possibilities, but we consider the latter to be the more plausible explanation since four different anti-H-2.23 antisera,

Fig. 2 SDS-PAGE of lymphoid cell antigens isolated with rabbit anti-rat $\beta_2\text{-}\mu$. 100 μl of an extract of either *a*, N.C3H splenocytes or *b*, 6C3HED lymphoma cells was reacted with 20 μl of rabbit anti-rat $\beta_2\text{-}\mu$ antiserum which had been prereacted with either 200 μg of BSA (●) or 100 μg of purified $\beta_2\text{-}\mu$ (○).

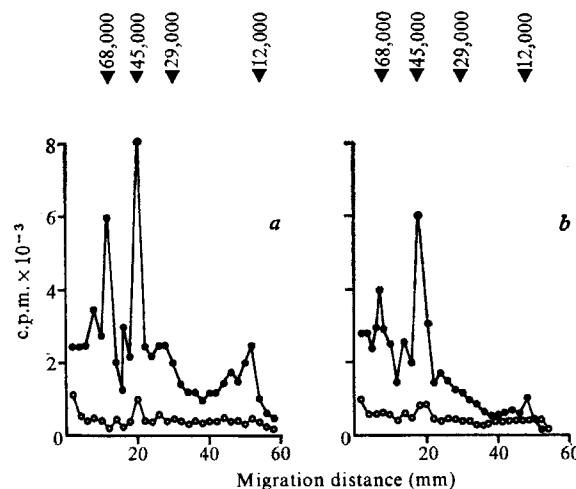
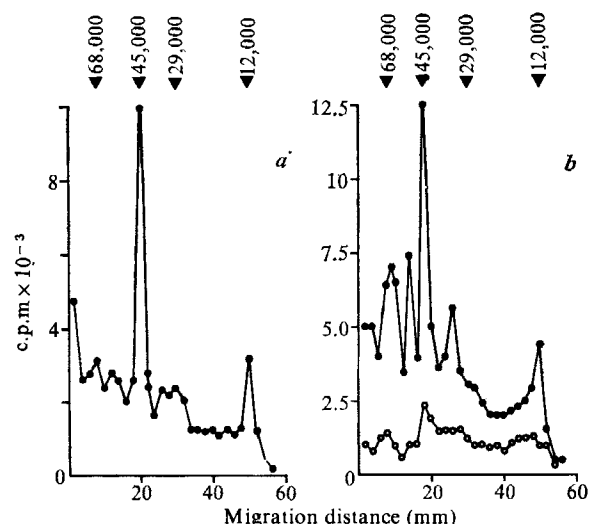


Fig. 3 SDS-PAGE of murine lymphoid cell antigens: effect of pretreatment with immunoadsorbent. Extracts of 6C3HED lymphoma cells were incubated overnight with an immunoadsorbent (100 μl adsorbent per 100 μl extract) prepared by coupling the crude Ig fraction of either rabbit anti-rat $\beta_2\text{-}\mu$ (○) or normal rabbit serum (●) to CNBr-activated Sepharose 4B to a final concentration of ~ 10 mg protein per ml beads. The adsorbent was removed and the supernatant incubated with either *a*, anti-H-2.23 (K603) or *b*, rabbit anti-rat $\beta_2\text{-}\mu$.

including (C3H.OH $\times$ B10.129) against C3H [K603], (B10 $\times$ LP.RIII) against B10.A(2R) [K358], (B10.RIII (7INS) $\times$ DBA/2) against B10.A [K323] and (B10.D2 $\times$ A) against B10.A (5R) [D23], gave identical patterns of reactivity in SDS-PAGE analysis of immunoprecipitates of radiolabelled 6C3HED cell extracts. It seems unlikely that all of these antisera would contain precisely the same contaminating antibodies. In addition, preliminary results indicate that anti-H-2 antisera which have been absorbed with normal C3H splenocytes are completely unreactive with extracts of 6C3HED cells. This suggests that the anti-H-2 antisera do not contain additional antibodies which are reactive with materials present only on the tumour cells.

Henning *et al.*¹³ have shown that viral antigens are present in close association with H-2 antigens on the surface of EL-4 leukaemia cells. Furthermore, Fujimoto *et al.*¹⁴ have shown that H-2 is associated with a tumour-associated antigen in the sera of lymphomatous A/J mice and Gooding and Edidin¹⁵ have shown that H-2 is physically linked to an additional antigenic moiety on the surface of teratoma cells. We have presented evidence which strongly suggests that the H-2- $\beta_2\text{-}\mu$ dimer is associated with additional proteins on the surface of 6C3HED lymphoma cells. Furthermore, syngeneic anti-tumour antisera, which are unreactive with normal C3H cells, precipitate the same material from tumour cell-extracts that is reactive with anti-H-2.23 (to be published elsewhere). These data suggest that a new complex is formed on the surface of these tumour cells and that this complex contains not only H-2 and $\beta_2\text{-}\mu$ antigenic determinants but also tumour-associated antigens.

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Anti-idiotypic antibodies against anti-vitamin A transporting protein react with prealbumin

IDIOTYPIC determinants on antibodies are those antigenic determinants in one antibody population which are not present in another antibody population from the same animal¹. The restriction of a set of idiotype determinants to antibodies directed against a single antigen suggests that the idiotype determinants may reside in the antibody combining site. In fact, amino acid sequence determinations of antibodies sharing the same idiotype have shown that the variable portions of light chains as well as heavy chains of the various antibodies are very similar if not identical (see ref. 2 for review). These and similar observations have suggested that antibodies against the idiotype determinants (anti-idiotypic antibodies) recognise only a clonotype, that is, antibodies with a given set of variable regions. This seems well founded in as much as antibodies raised in two species against a single antigen only infrequently seem to share idiotype determinants. Since the antibody system is degenerate then some antibodies raised against a protein antigen, called A, which forms part of the protein-protein complex AB, may recognise protein A in a fashion similar to that of the normally interacting protein B. Consequently, the idiotype determinants of some of the antibodies against protein A may have structures in common with protein B. If so, antibodies raised against such idiotype determinants should bind to protein B. To test this assumption we explored the situation with regard to the retinol-binding protein (RBP)-prealbumin protein complex³⁻⁶. The data presented here show that it is possible to raise anti-idiotypic antibodies against anti-RBP and that such antibodies interact with prealbumin.

Human RBP and prealbumin were isolated as described previously³. Various chemical, physical-chemical, and radio-immunological tests revealed that both proteins were free from cross-contamination (for details see ref. 7). Rats were immunised with RBP⁸ and after several booster injections the rats were exsanguinated and the serum collected. Sera from 10 rats were pooled to obtain sufficient amounts for further processing. Antibodies against human RBP were isolated by immunosorbent purification on a Sepharose 4B column to which RBP had been covalently attached⁹. The bound antibodies were desorbed with 0.2 M glycine-HCl buffer pH 2.9. Although RBP which has been subjected to acid does not interact with prealbumin⁹ it was important to ascertain that the RBP antibody preparation did not contain any rat prealbumin. To this end the antibody preparation was labelled¹⁰ with ¹²⁵I and immunoprecipitated with a specific anti-rat prealbumin serum by a sandwich procedure¹¹.

Although this test did not reveal the presence of any prealbumin it is likely that if prealbumin represented less than about 0.002% of the total protein its presence would have escaped detection. Furthermore, heavily overloaded SDS-polyacrylamide gels¹¹ did not reveal any protein zones which could be identified as prealbumin after electrophoresis; more than 98% of the antibody preparation labelled

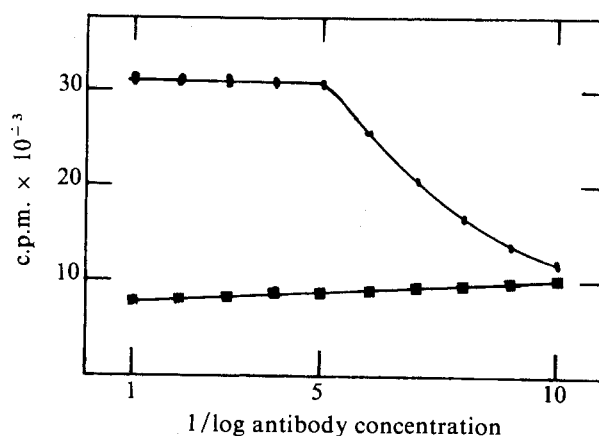


Fig. 1 Binding of anti-idiotypic antibodies against anti-RBP to ¹²⁵I-labelled prealbumin. The anti-idiotypic antibody preparation (initial concentration 2 mg ml⁻¹) was serially diluted and mixed with ¹²⁵I-labelled prealbumin (32,000 c.p.m.). The resulting immune complexes were collected by the use of protein A-containing *Staphylococcus aureus*¹¹. As a control serial dilutions of preimmune rabbit IgG of the same concentration as the anti-idiotypic antibodies was used. ●, Anti-idiotypic antibodies; ■, preimmune IgG.

with ¹²⁵I was precipitated by a rabbit anti-rat IgG serum; and immunoelectrophoresis of the anti-RBP preparation revealed a single precipitin line with a polyvalent rabbit anti-rat serum protein serum. All of these criteria, and others which we shall describe, suggested that the isolated rat anti-RBP antibodies were of high purity.

The specific rat antibodies against RBP were injected into a rabbit which was repeatedly challenged with the antibodies over a period of 3 months. Theoretically, the rabbit antiserum contained antibodies against the common portions of rat immunoglobulin as well as antibodies to the particular idiotype determinants. The IgG of the rabbit serum was isolated on a goat anti-rabbit IgG-Sepharose 4B immunosorbent column. To remove antibodies against the common rat IgG determinants the rabbit IgG fraction was passed over an immunosorbent column of Sepharose 4B containing covalently bound normal rat IgG. Material which passed the column unretarded did not bind to ¹²⁵I-labelled normal rat IgG but reacted with ¹²⁵I-labelled rat antibodies against RBP. Thus, the rabbit antibodies, isolated as described, were apparently specific for idiotype determinants present on rat antibodies against RBP.

To test whether the anti-idiotypic antibodies against anti-RBP would react with human prealbumin, the latter protein was labelled with ¹²⁵I and mixed with serial dilutions of the anti-idiotypic antibodies. The resulting immune complexes were collected as described¹¹. In a control experiment the anti-idiotypic antibodies were replaced by preimmune rabbit IgG. Figure 1 shows that the anti-idiotypic antibodies reacted with prealbumin. In all tests performed the anti-idiotypic antibodies could bind more than 90% of the labelled prealbumin. Preimmune rabbit IgG did not react with prealbumin (Fig. 1), suggesting that the reaction was specific.

The titre of the anti-idiotypic antibodies was low; 1 ml of antiserum could bind 160 ng of human prealbumin and 120 ng of rat prealbumin. One ml of a regular rabbit antiserum against rat prealbumin bound 150 µg of rat prealbumin but only 10 ng of human prealbumin. These observations show that the immunological cross-reactivity between human and rat prealbumin, as for RBP¹², is low and, thus, the data are consistent with the view that the rabbit anti-anti-RBP serum did not contain any antibodies that had been raised against prealbumin directly.

If the antibodies reactive with prealbumin are genuine

anti-idiotypic antibodies against anti-RBP the binding between prealbumin and these antibodies would be abolished in the presence of antibodies against RBP. To examine this ^{125}I -labelled human prealbumin and anti-idiotypic antibodies against anti-RBP were mixed together with serial dilutions of two rat IgG fractions. One fraction contained rat antibodies against RBP whereas the other fraction comprised only preimmune IgG from the same rats. Figure 2 shows that preimmune rat IgG did not affect the reaction between ^{125}I -labelled prealbumin and the anti-idiotypic antibodies. This reaction was completely inhibited in the presence of rat antibodies against RBP, however.

The same experiment was repeated with a regular rabbit antiserum against rat prealbumin substituting for the anti-idiotypic antiserum. In this case neither rat anti-RBP nor preimmune rat IgG had any effect on the binding of the prealbumin-antibodies to prealbumin (not shown). These data give strong support to the view that it was the anti-idiotypic antibodies against RBP which recognised prealbumin.

Since there is no apparent immunological cross-reactivity between RBP and prealbumin⁸ it seemed likely that the anti-idiotypic antibodies against RBP recognised the RBP-binding site on the prealbumin molecule. If so, RBP should abolish the interaction between the anti-idiotypic antibodies and prealbumin. To examine this various amounts of RBP were added to a mixture of ^{125}I -labelled prealbumin and the anti-idiotypic antibodies. As a control RBP was replaced by soy bean trypsin inhibitor. Figure 3 shows that RBP, but not soy bean trypsin inhibitor, completely abolished the interaction between prealbumin and the anti-idiotypic antibodies. This suggests that the anti-idiotypic antibodies bound to the RBP-binding site on prealbumin.

To explain why anti-idiotypic antibodies raised against anti-RBP interact with prealbumin it seems reasonable to assume that a portion of the antibodies raised against RBP recognises this protein in a way similar to the way prealbumin recognises RBP. Due to the degeneracy of the antibody system, some of the combining sites of the antibodies may in fact be very similar to those structures of

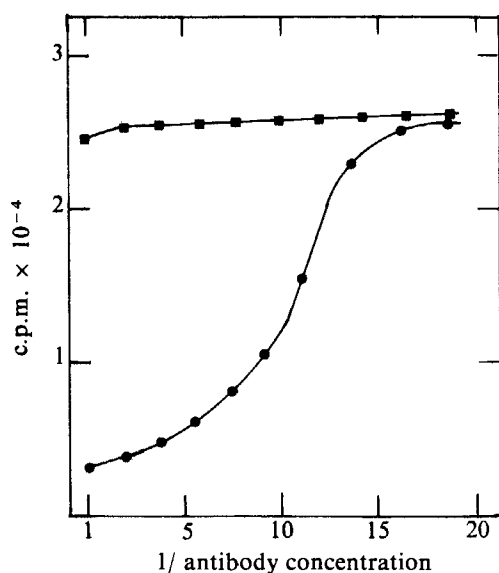


Fig. 2 Competition for binding to the anti-idiotypic antibodies against anti-RBP between prealbumin and rat antibodies against RBP. ^{125}I -labelled prealbumin (30,000 c.p.m.) was mixed with enough anti-idiotypic antibodies to cause 80% binding and serial dilutions of specific rat antibodies against RBP (initial concentration 0.5 mg ml^{-1}). As a control the rat antibodies were replaced by preimmune rat IgG at a concentration identical to that of the RBP antibodies. Immune complexes were precipitated as described before¹¹. ●, Rat antibodies against RBP; ■, pre-immune rat IgG.

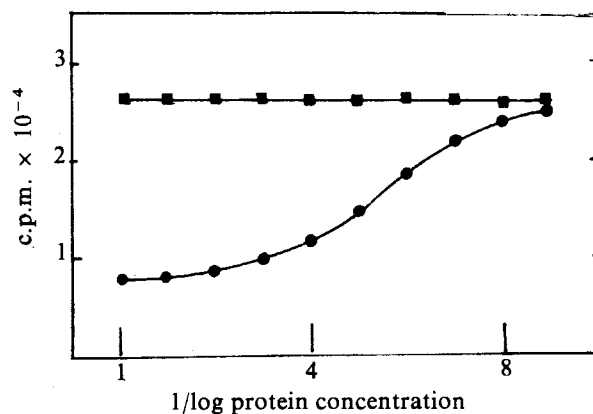


Fig. 3 Competition for binding to prealbumin between RBP and anti-idiotypic antibodies against anti-RBP. ^{125}I -labelled prealbumin (29,000 c.p.m.) was mixed with enough anti-idiotypic antibodies to ascertain 80% binding and serial dilutions of highly purified RBP (initial concentration 0.25 mg ml^{-1}). As a control soy bean trypsin inhibitor was used instead of RBP. Immune complexes were collected as described¹¹. ●, RBP; ■, soy bean trypsin inhibitor.

prealbumin which binds to RBP. If so, a second set of antibodies raised against such antigen-combining sites would cross-react with prealbumin. The reaction with prealbumin should be confined only to that part of the molecule which binds to RBP. This is indeed the finding since RBP competes efficiently with the anti-idiotypic antibodies for binding to prealbumin despite the fact that antibodies raised directly against prealbumin will react with this protein regardless of whether RBP is bound or not⁸.

Only a subset of the anti-idiotypic antibodies would recognise prealbumin since RBP displays multiple antigenic sites⁸. In fact, RBP interacts with a cell-surface receptor with a site that is distinct from the prealbumin-binding site^{4,12}. Another subset of anti-idiotypic antibodies against anti-RBP abolishes the interaction between RBP and the cell surface receptor (K.S. and P.A.P., in preparation).

If generalised, the method described here to raise antibodies reactive with a protein which never was used in the immunisation procedure would be useful in obtaining antibodies against interacting proteins when only one of the proteins is available. Consequently anti-idiotypic antibodies against insulin would react with the insulin receptor. Preliminary observations in our laboratory suggest that this may indeed be the case.

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Transient increase in muscarinic acetylcholine receptor and acetylcholine sterase in visual cortex on first exposure of dark-reared rats to light

AN organised sequence of cellular events, some transient, others more lasting, occurs in the visual system on first exposure of dark-reared rats to the light. Hitherto these changes have been biochemically characterised only in terms of rates of change of precursor uptake into protein fractions or *in vitro* assayed enzyme activities. We have shown that, in the visual cortex, the onset of visual experience is accompanied by a modulation of the total functional quantities of two classes of macromolecule with reasonably well understood functions in the cellular economy. One is the colchicine-binding microtubular protein, tubulin¹. We report here rapid transient changes in the amount of a protein known to be involved in synaptic functions, the muscarinic cholinergic receptor (mAChR), on the onset of light.

We showed previously that there is an elevation in the synthesis or turnover of several protein fractions in the visual cortex, as well as the lateral geniculate and retina^{2,3}. In the cortex, much of this increase is associated with a rapidly labelling and exported neuronal (glyco-)protein fraction⁴ whose transport is blocked by colchicine⁵. The microtubule system is apparently involved, since within 1 h of the onset of light, incorporation of ³H-lysine into a polymerisable tubulin fraction doubles⁶ and within 3 h there is a 23% elevation in the amount of colchicine-binding protein (largely tubulin) in the visual cortex¹. Over the same period, there is an increase in the activity of several acid hydrolases of up to 30%⁷.

All the changes listed above seem to be lasting, in that they persist for longer than 24 h and/or are present when dark-reared animals are compared to normally-reared littermates, with the exception of the elevation in colchicine-binding, which does not last beyond 24 h of light exposure. However, we have also found presumptive evidence for some apparently transient increases in the activity of the enzymes of acetylcholine metabolism, choline acetyltransferase (CAT) and acetylcholinesterase (AChE), in that while dark-reared animals did not differ in visual cortex enzyme activity from their normally-reared littermates, 3 h of light exposure of the dark-reared animals did produce a 30% elevation of activity of both enzymes⁷.

The transmitter(s) involved in the mammalian visual system are not known for certain, and the mere presence of relatively high concentrations of ACh and its enzymes is not, in itself, sufficient evidence for a transmitter role. However, the recent development of techniques for the detection and measurement of specific receptor binding proteins have shown the presence of significant quantities of a muscarinic cholinergic receptor at cortical synapses⁸. It has therefore become possible to examine the effects of dark rearing and visual experience on a molecule which may play a key part in the inter-neuronal transmission machinery.

Male Wistar rats were born and maintained in the dark until 50–55 d of age, at which time half of each litter was exposed, in individual cages, to normal laboratory illumination for 3 or 24 h (light exposed, L); the remainder were held in the dark, also in individual cages (dark controls, D). To avoid complications due to diurnal rhythmicity (N. Wood and S.P.R.R., in preparation) all exposures were carried out during the morning. In some experiments, a proportion of the littermates were removed from the dark at weaning (21 d) and subjected to a normal animal house

environment (12 h light, 12 h dark) until 50 d (normals, N). Experiments comparing L and D, D and N, and, in the AChE experiments, L, D and N groups, were made. Rearing conditions were exactly as described previously^{1,3}. Following treatments, L, D and N animals were killed, visual and motor cortex samples removed from each and homogenised in 0.05 M Na-K phosphate buffer, pH 7.4. Homogenates were either assayed directly or frozen until used. Preliminary experiments showed that freezing and thawing up to three times had no effect on the parameters to be measured.

Portions of each homogenate were assayed for: (1) muscarinic cholinergic receptor binding; (2) acetylcholinesterase; and (3) protein. Measurement of the muscarinic binding was based on the 3-quinuclidinylbenzilate (QNB) method of Yamamura and Snyder⁹ (see Table 1). Acetylcholinesterase (EC 3.1.1.7) was measured by the method of Ellman *et al.*¹⁰ and protein by that of Lowry *et al.*¹¹. Balanced groups were used in each experiment and batch variation in the mean QNB binding or AChE activity between experiments was eliminated by standardising results between batches. Statistical comparisons between groups were made using a two-tailed Student's *t*-test.

Figure 1 compares QNB binding in visual and motor cortex in 3- and 24-h light-exposed animals with that in their dark control littermates. At 3 h after the onset of light exposure, there was a 54% increase in QNB binding in the visual cortex of L compared with D animals ($t = 2.89$, $P < 0.01$). In the L animals, QNB binding in the visual cortex was 46% greater than in the motor cortex ($t = 2.33$, $P < 0.05$). There were no significant differences between binding in the visual and motor cortex of D animals, or between L and D animals in the motor cortex. However, the enhancement of QNB binding in the visual cortex of L animals was transient, as by 24 h binding activity had returned to the control level. A further indication that the effect was a transient response to the onset of light stimulation is given when QNB binding is compared in D and N animals (Table 1). There were no differences between animals in the two conditions, so light exposure cannot be necessary for the long-term normal development of cortical mAChR.

AChE activity was then measured in the same tissue samples. There were no differences in motor cortex levels

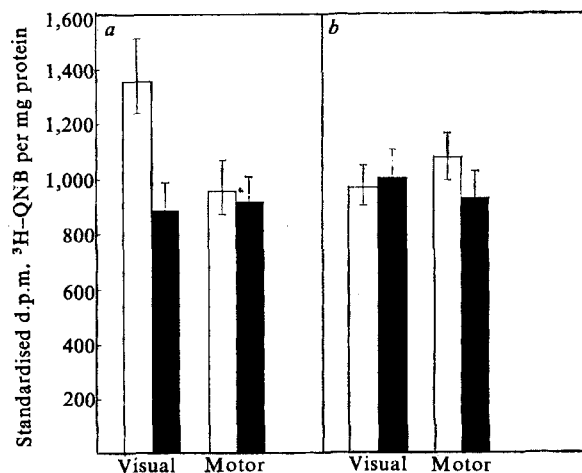


Fig. 1 ³H-QNB binding (black bars) was determined (open bars) in visual and motor cortices of dark-reared and light-exposed littermates aged 50–55 d as described in Table 1. Light exposure was for 3 h (a) or 24 h (b). Results are means of ≥ 16 animals in each case; error bars show s.e.m. Significance values are given in text. Results are expressed as standardised ³H-QNB radioactivity such that 1,000 d.p.m. $\approx$ 1.0 pmol QNB bound per mg protein, but 3- and 24-h experiments are not directly comparable.

at either time, or between D and N animals in the visual cortex. There was a 24% elevation in visual cortex specific activity in L compared with D, and 18% in L compared with N, and both differences are significant (L versus D, $t = 3.94$, $P < 0.001$; L versus N, $t = 2.70$, $P < 0.02$). However, by 24 h the AChE activity in the L visual cortex had declined towards the controls and the difference is no longer significant ($t = 0.45$). The elevation of L over D at 3 h replicates our previous observation⁷ and the decline at 24 h confirms the prediction made in that paper.

These experiments, together with our earlier ones, demonstrate that the activities of the three components of the acetylcholine transmitter system in the visual cortex are responsive to the onset of visual input in dark-reared animals. CAT⁷, AChE and mAChR all show an elevation of specific activity of 24–54% within 3 h of the onset of light exposure; however, the response is transient, and by 24 h has disappeared.

These observations have two sets of implications. The first relates to understanding the mechanisms whereby experiential events (including learning) modulate neuronal connectivity. It is known that in the rodent, visual experience results in long-term changes in dendritic branching, spine formation and synaptic number and density^{12–14}. While enhanced protein synthesis and turnover of microtubular protein may underly these morphological changes^{2,4,6}, it seems that they do not involve a permanent increase in the activity of the ACh transmitter system per mg cortex protein. However, it is also well known that the fixation of experience in the brain is at least a two-stage process, the first being labile, the second more lasting, and that these stages can be uncoupled by the selective use of biochemical inhibitors (see, for example, ref. 15). Mobilisation of CAT–AChE–mAChR system could represent an aspect of synaptic potentiation associated with the labile phase of the fixation of experience. It is important to note that, unlike the enzyme activity measurements, those of mAChR involve a more direct assay of the number of

receptor molecules, or binding sites, present.

The second question raised by our observations relates to their molecular basis. Enhanced enzyme or binding activity could result from an activation or unmasking of pre-existing sites, or from a temporary increase in the *de novo* synthesis of the enzymes and receptors. To distinguish between these alternatives would provide evidence as to the nature of the control processes involved. Experiments to test this, and to clarify the subcellular locus of the enhanced QNB binding, are in progress.

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Table 1 ³H-QNB binding in visual and motor cortex of dark-reared and normal littermates

Condition	Standardised ³ H-QNB binding (d.p.m./mg prot.) in:— visual cortex	motor cortex
Normals	983 ± 78 (16)	1,011 ± 107 (16)
Dark-reared	871 ± 125 (13)	923 ± 60 (12)

Aliquots of homogenate containing between 50 and 120 µg protein were incubated in a reaction mixture containing 52 pmol. ³H-QNB, specific radioactivity 8–8.9 Ci mmol⁻¹ (Radiochemical Centre, Amersham) in the presence or absence of 125 nM atropine and 0.05 M Na-K phosphate buffer, pH 7.4, to a final volume of 2.0 ml. Incubations were at 25 °C for 60 min and each sample was assayed six times, three times with, and three times without, atropine present. After incubation, the reaction mixture was filtered under suction through 2.5 cm Whatman GF/B filter disks and washed rapidly three times with 5 ml ice-cold Na-K phosphate buffer. The disks were dried for 1 h at 45 °C and counted in a toluene-phosphor scintillant containing 0.2% v/v Triton X-100. Specific QNB binding (p mol per mg protein) was calculated from the mean difference in bound counts in the absence and presence of atropine. Preliminary experiments confirmed the applicability of the assay to unfractionated tissue homogenates. Binding in the presence of atropine was generally some 50–60% of that in its absence and increasing the atropine concentration did not further reduce it. No artefactual atropine-sensitive binding to the filters in the absence of tissue occurred. The variability within triplicate assays of the atropine-sensitive binding was generally within the range ±15% and assays that fell outside these limits were discarded or repeated. The assay was linear with increasing tissue concentration, and average binding, of 0.8–1.2 pmol QNB per mg tissue protein, was similar to that reported by Yamamura and Snyder⁸. Binding was determined in visual and motor cortices of dark- and normally-reared littermates aged 50–55 d. Values are ± s.e.m. with the number of animals tested shown in parentheses. There are no significant differences between any group. Standardisation is such that 1,000 d.p.m. ≈ 1.0 pmol QNB per mg protein.

Rapid accumulation of high molecular weight acetylcholinesterase in transected sciatic nerve

NERVOUS tissue and muscle in rat^{1–4} and chicken^{5,6} contain several molecular forms of acetylcholinesterase (EC 3.1.1.7, AChE), distinguishable by their sedimentation coefficient in sucrose gradient⁷. In rat, several peripheral nerves and non-innervated regions of skeletal muscle^{1,2} were shown to contain two molecular forms of AChE, with respective sedimentation coefficients of 4 and 10S; in addition to these two forms, a high molecular weight form with a sedimentation coefficient of 16S was found in the innervated regions of various skeletal muscles^{1,2,4}. After denervation, the 16S form, which we will call the H form, either disappeared from skeletal muscle³ or was drastically reduced^{1,4}. In chicken, several nerves exhibited three molecular forms of AChE with sedimentation coefficients of 4, 6.5 and 11S, while various skeletal muscles contained an additional molecular form with a sedimentation coefficient of 19.5S (ref. 5). After denervation, the high molecular weight form 19.5S, which by analogy with the 16S of rat is referred to here as the H form, disappeared from crude extract of muscles⁵. As the H form was only found in tissues innervated by cholinergic nerve endings and disappeared or decreased drastically from denervated skeletal muscles, it has been suggested that this form of AChE is exclusively myogenic^{1,3} and may constitute the specific endplate enzyme^{1,2,4,5}. We report here that tiny amounts of the H form of AChE can be detected in crude extracts of intact sciatic nerves of rat and chicken, and that after transection of the sciatic nerve with the ensuing blockade of the axonal traffic, the H form increased rapidly at the site of injury.

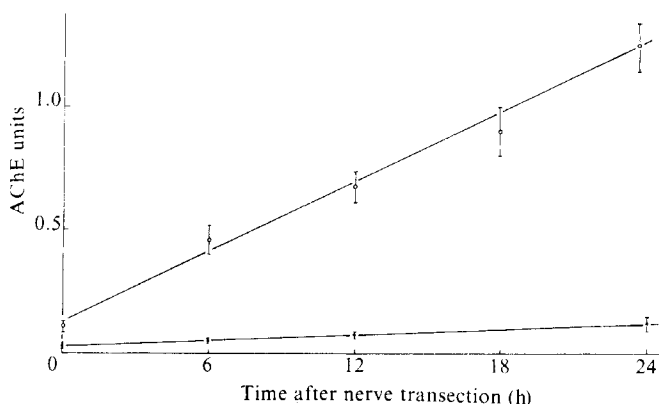


Fig. 1 AChE accumulation in transected sciatic nerve of rat (●) and chicken (○). Nerves were transected with scissors near the peroneal-tibial branching in rat and the ischiatic-femoral branching in chicken. At 6, 12, 18 and 24 h after transection, animals were killed and a 2-mm segment was cut off the tip of the proximal stump. The segment was homogenised in 1 M NaCl, 0.01 M Tris-HCl pH 7, 0.05 M MgCl₂ and 1% Triton X-100, and centrifuged at 20,000g for 20 min at 4 °C. The AChE was assayed in the supernatant at 20 °C, by the method of Ellman *et al.*¹² with acetylthiocholine as substrate and 10⁻⁴ M ethopropazine as pseudocholinesterase inhibitor. One unit corresponds to hydrolysis of 73.5 nmol acetylthiocholine per min (ref. 13). Three animals per time-point were used.

Young Leghorn chicks (4 weeks old) and Wistar rats (2 months old) were anaesthetised with chloroform and one sciatic nerve was intersected by cutting out a 2-mm segment for use in measuring AChE concentrations in intact nerves (see legend to Fig. 1). At various intervals after transection (Fig. 1) the animals were killed and 2-mm segments cut off the end of the proximal stump. Nerve segments were then homogenised and the amount of AChE in the homogenate was determined (see legend to Fig. 1). Homogenates were then sedimented

through sucrose gradient to allow separation and characterisation of the different molecular forms of AChE (legend to Fig. 2).

In intact sciatic nerve of rat and chicken, AChE concentrations were respectively 10 and 55 mU mm⁻¹ (for definition of unit see Fig. 1 legend). In both rat and chicken, separation procedure revealed the presence of four molecular forms of AChE (Fig. 2, 0 h). In rat sciatic nerve, we found a tiny amount (1.6%) of the H form (16.5S), one major form of 10S (M) which accounted for 63% of total AChE, and two low molecular weight forms (L) with respective sedimentation coefficients of 4S (L₁) and 6.5S (L₂), which accounted for 28 and 7.4% of total AChE. In chicken sciatic nerve, the H form (20S) only accounted for 1.3% of total AChE; the M form (73%) had a sedimentation coefficient of 11.5S; the two L forms had sedimentation coefficients of 5S (L₁) and 7S (L₂) respectively and accounted for 6 and 20% of total AChE. The presence of such small amounts of the H form in sciatic nerve of rat and chicken might explain why this form was not previously detected in nerve extracts^{1,2,3}. Detection of the H form, as well as of the 6.5S form in rat, which had also not been seen previously^{1,2}, was possible because of the higher resolution attained with the SW 41 Beckman rotor.

AChE activity in the 2-mm terminal segment from the proximal stump of the transected sciatic nerve, increased linearly during the 6th and 24th hours after transection (Fig. 1), at a rate of 4.2 mU h⁻¹ in rat and 47 mU h⁻¹ in chicken. To identify the molecular forms of AChE responsible for this rise in AChE activity, the crude extracts of the 2-mm segment were analysed by sedimentation in sucrose gradient. The sedimentation profile of AChE activity showed that only the activity of the two high molecular weight forms M and H increased with time, while that of the two low molecular weight forms L₁ and L₂ either remained unchanged or increased slightly (Fig. 2). The rise in the activity of the H form was very striking: 6 h after nerve transection, its concentration had increased 15-fold in rat and 20-fold in chicken (Fig. 3). For the M form, the rise was comparatively less pronounced, even though this form was responsible for most of the total increase in AChE activity,

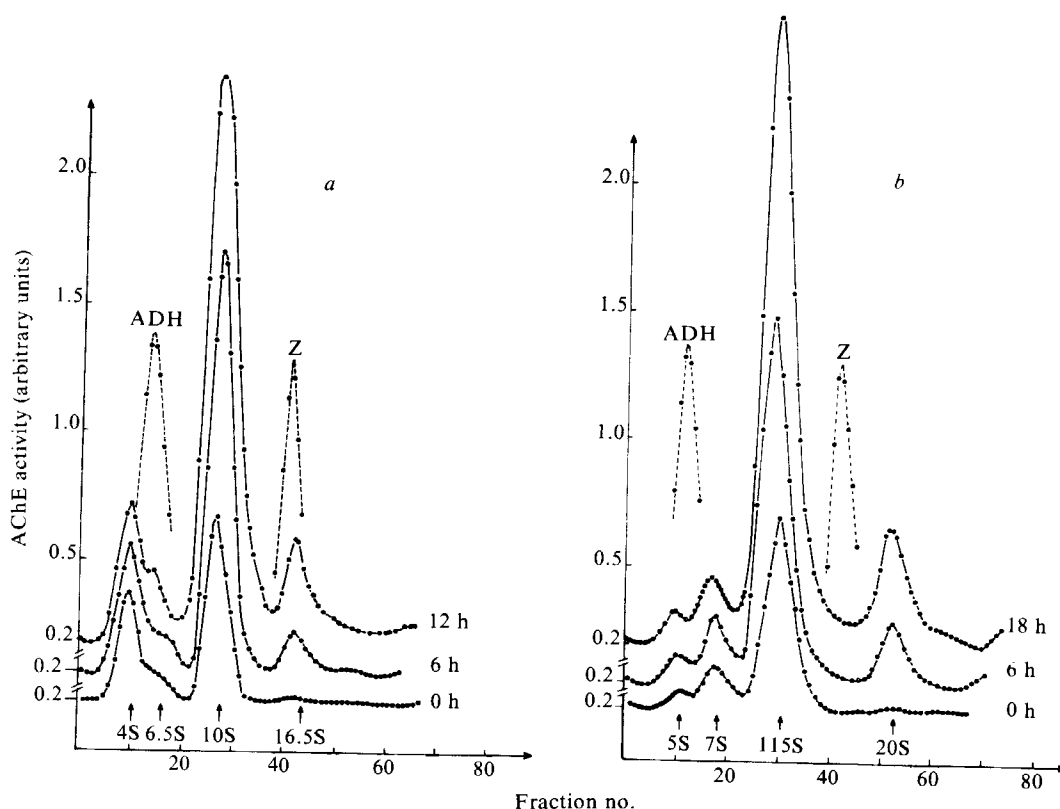


Fig. 2 AChE sedimentation profiles in intact (0 h) and transected sciatic nerves of rat (a) and chicken (b). The 20,000g supernatant (see Fig. 1) was mixed with two markers: ADH (alcohol dehydrogenase, 4.8 S, Boehringer) and Z (β-galactosidase, 16S, Boehringer), layered on a 5-20% sucrose gradient and centrifuged in a SW 41 rotor (Beckman). Approximately 70 fractions were collected. Each fraction was assayed for AChE, ADH and Z.

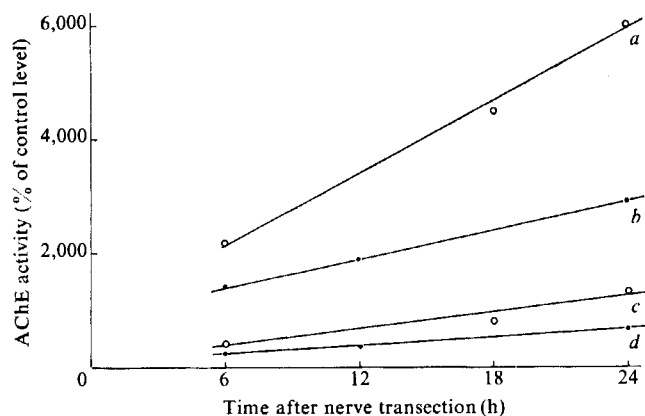


Fig. 3 Accumulation kinetics for the high molecular weight forms of AChE. The rise in the activity of each form at the 2-mm tip of the proximal stump is expressed as % of the same form's activity in the intact nerve (see Figs 1 and 2). *a*, Chicken, 20S; *b*, rat, 16S; *c*, chicken 11.5S; *d*, rat, 10S.

thus, 6 h after transection, M-form activity had doubled in the chicken and increased 1.5 times in the rat (Fig. 3).

In short, both rat and chicken sciatic nerves contain four molecular forms of AChE—two high molecular weight forms (H and M) and two low molecular weight forms (L_1 and L_2). After transection of the sciatic nerve, only the high molecular weight forms accumulated rapidly at the end of the proximal stump, whereas the low molecular weight forms either remained stationary or accumulated very slowly.

Previous work on dog⁸, cat⁹ and rats^{10,11} showed that after transection or ligation of the sciatic nerve, AChE accumulated at both sides of the lesion, at the speed of rapid axonal transport^{8,9,11}. In dog and cat, it was estimated that approximately 15% of the AChE present in the sciatic nerve was transported fast (10% outward and 5% inward), while the rest was either stationary or slow-moving^{8,9}. In rat, only 3% of the AChE in the sciatic nerve was transported fast¹¹.

Our results therefore indicate (1) that the H form of AChE is present in the intact nerve of rat and chicken and (2) that this and the M form (the other high molecular weight form) move with the rapid phase of axonal transport, whereas the low AChE molecular weight forms L_1 and L_2 , also present in the intact nerve of rat and chicken, either remain stationary or move with the slow phase of axonal transport.

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Cholinesterase is associated with the basal lamina at the neuromuscular junction

THE cholinesterase (ChE) of skeletal muscle is concentrated at the neuromuscular junction, where it hydrolyses acetylcholine released from the nerve terminal^{1–3}. In frog muscle treated with a histochemical stain for ChE, enzyme activity is demonstrable at all terminal branches of the neuromuscular junction (Fig. 1*a*), with the reaction product occupying the synaptic cleft between nerve and muscle (Fig. 1*d*)^{4,6}. A particulate enzyme, ChE was assumed to be integral to the postsynaptic plasma membrane³ until Hall and Kelly⁷ showed that mild protease treatment of intact muscles released active ChE into the medium without apparent damage to the plasma membrane. One possibility raised by this result is that ChE is associated with the basal lamina (BL; sometimes called basement membrane) that runs through the synaptic cleft⁸. This idea was supported by the finding that a subunit of ChE from electric organs of fishes and rays has several properties in common with collagen^{9–11}, a protein known to be a principal component of BLs¹². Here, we present direct evidence that junctional ChE is associated with the BL of the synaptic cleft in skeletal muscle.

Our approach was to remove the cellular components of the neuromuscular junction *in vivo* (nerve terminal, Schwann cell, and muscle cell; Fig. 1*d*) leaving behind the BL; we then assayed ChE histochemically. The nerve to the cutaneous pectoris muscle of the frog was cut, causing degeneration of the nerve terminals¹³. A long portion of nerve was removed to maintain denervation, inducing Schwann cell processes to retract from synaptic areas¹³. Muscles were injured mechanically (see Fig. 1 legend and ref. 14), causing disruption of muscle fibres; the damaged cells decomposed, leaving behind the sheaths of BL that surrounded them^{14,15}. Frogs were X-irradiated to prevent regeneration of new muscle within the BL sheaths (ref. 16 and J.R.S., L.M.M. & U.J.M., in preparation). Cellular debris and membrane fragments within the sheaths were phagocytosed^{14,15} and electron microscopy showed that nearly all (98.3±0.4%; mean±s.e.m. of samples from 41 sheaths) of the surface area of the BL was clean by two weeks after the muscle was damaged. Portions of such sheaths are shown in Fig. 1*c*.

When we stained such preparations for ChE^{8,13,17} and examined them under the light microscope, bands of reaction product (Fig. 1*b*) corresponding in size, shape and arrangement to the ChE-outlined terminal arborisations of normal muscle (Fig. 1*a*) were revealed. In addition, the bands had a periodic substructure corresponding to that of the junctional folds (Fig. 1*d*), which are arranged at intervals of approximately 1 μm along the endplate⁸. (The periodic variation in staining intensity is particularly prominent after the overlying nerve terminal and Schwann cell processes are removed by denervation, whether the muscle is damaged or not¹³.) Thus, staining for ChE showed that the enzyme was concentrated at junctional sites on the sheaths.

Electron microscopy of the sheaths confirmed that ChE was associated with BL. Reaction product was concentrated in and near the BLs that had occupied the synaptic cleft and projected into junctional folds (Fig. 1*e*). Because reaction product might have obscured remnants of the muscle at junctional sites, we examined preparations not stained for ChE, using the BLs from junctional folds and Schwann cells (Fig. 1*e*) to identify junctional sites on the sheaths¹⁴. Less than 2% of the junctional BL (1.8±0.5%; *n*=28) was apposed by fragments of plasma membrane and this is far too little to account for the even pattern of

ChE staining seen light and electron microscopically.

Light microscopic observations provided additional information about the ChE associated with BL. ChE-stained bands on the BL sheaths were fainter than those on normal fibres; it is known that levels of junctional ChE decrease following denervation^{1,2,13,16}. ChE-stained bands on the sheaths were similar in intensity to those on denervated,

but undamaged, fibres in the same preparations; thus, a major fraction of the ChE that survived denervation remained with the BL after the muscle was removed. The intensity of staining was reduced by BW 284C51, a specific inhibitor of 'true' acetylcholinesterase (EC 3.1.1.7) (ref. 2), demonstrating that some, if not all, of the ChE in the BL is acetylcholinesterase.

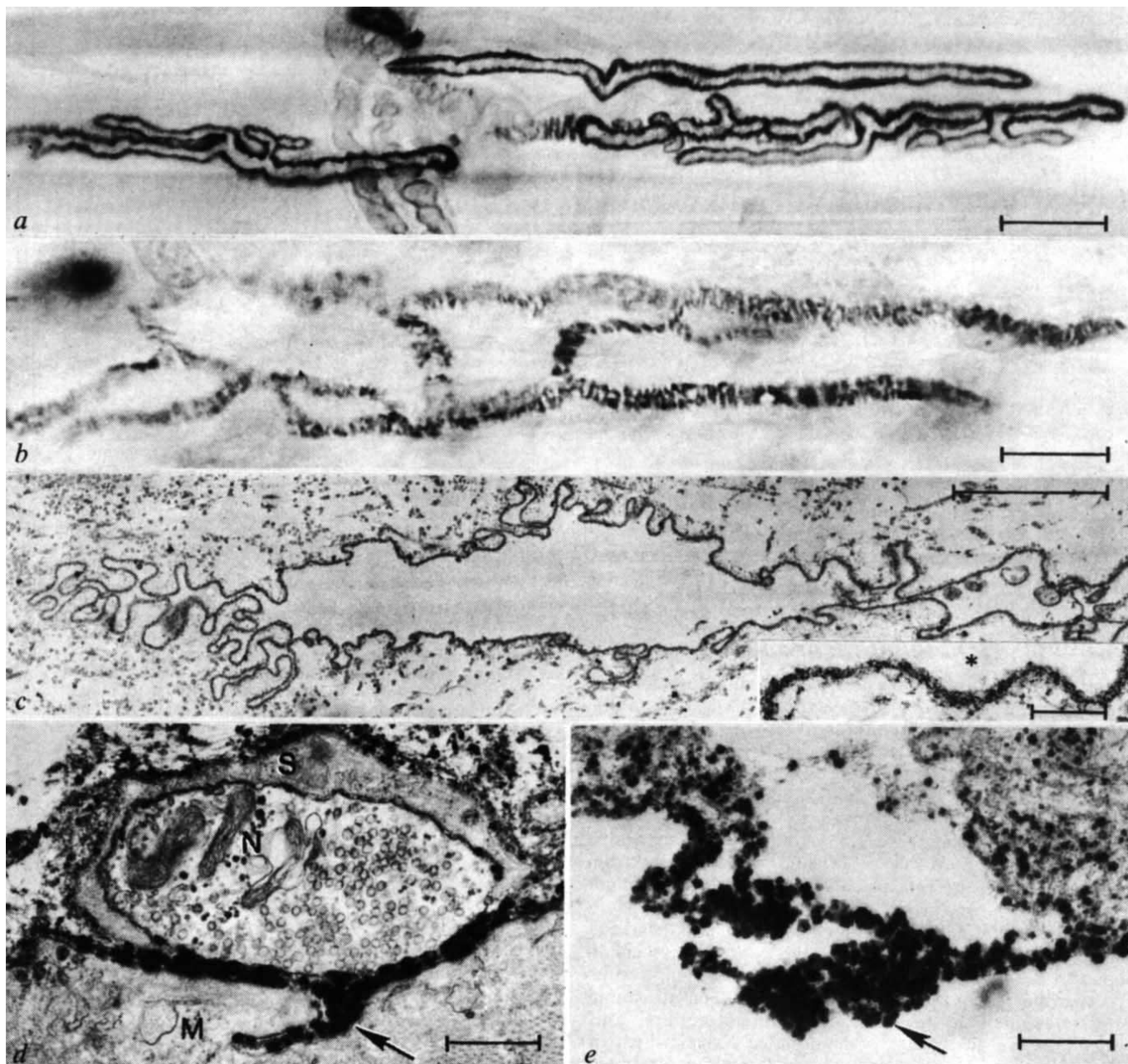


Fig. 1 *a, b*, Light micrographs of ChE-stained endplates (sites of neuromuscular junctions) on a normal muscle fibre (*a*) and on a BL sheath that survived denervation and muscle damage (*b*). Bars represent 20 μ m. *c*, Low-power electron micrograph of a cross section through a BL sheath. Collagen fibrils lie outside the BL and the inside, once occupied by the muscle fibre, is now nearly empty. Bar represents 2 μ m. Inset: high-power micrograph of a portion of a BL sheath demonstrates the absence of muscle cell plasma membrane which is normally situated 10–20 nm from the BL. Asterisk is in the lumen of sheath. Bar represents 0.2 μ m. *d, e*, Electron micrographs of ChE-stained junctional sites from preparations similar to those shown in *a* and *b*, respectively. Muscle cell (M), Schwann cell process (S), and nerve terminal (N), all present at the normal neuromuscular junction (*d*), disappear following denervation and muscle damage (*e*), leaving behind the BL that surrounded muscle and Schwann cell and projected into junctional folds (arrows). The muscle in *d* was stained lightly for ChE, to avoid obscuring cellular elements. Bars represent 0.5 μ m. All experiments were carried out on the cutaneous pectoris muscles of 5-cm male *Rana pipiens*. To prepare BL sheaths, frogs were submitted to the following regimen: On day 1, the nerve to the cutaneous pectoris was cut within 1 mm of the muscle border, and a 2-cm segment of nerve was evulsed. On day 12, pieces were cut from each end of the muscle, leaving behind a central row of 1–1.5-mm long muscle fibre segments, many of which bore endplate sites (details in ref. 14). On day 15, frogs were X-irradiated (250 kV; 15 mA; added filter of 2 mm Al; 65 cm from source to centre of animal; 100 rad min⁻¹; total dose, 1,600 rad). Lead shielding placed over the frog restricted radiation to the thorax. On day 26, animals were killed and muscles were anaesthetised with MS 222. Normal (*a, d*) and damaged (*b, e*) muscles were prepared for microscopy as described previously^{6,13}. Ruthenium red was used to stain BL for electron microscopy¹⁴. The histochemical procedure of Karnovsky¹⁷ was used to demonstrate ChE activity^{6,13}. Results similar to those illustrated here were also obtained with muscles that were denervated and damaged on day 1, irradiated on day 3, and fixed 3–5 weeks later.

Thus, at least some of the ChE at the frog neuromuscular junction is very tightly bound to (that is, contained in or connected to) the basal lamina of the synaptic cleft. To our knowledge, this is the first case in which an enzyme has been shown to be associated with the basal lamina of any cell.

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Effect of calcium ionophore Br-X537A on renin synthesis and release in *Amphiuma means* kidney culture

CALCIUM ions play an important part in stimulus-secretion coupling¹, and in particular have been implicated in the control of renin release from kidneys *in vitro*². The calcium ionophores A23187 and X537A and its derivatives have been used in several studies of the effect of mobilisation and movement of calcium and other divalent ions on biological systems, including mast cells, pancreatic B cells and the neurohypophysis^{3–8}. X537A and Br-X537A are of interest because they release calcium preferentially from intracellular sites irrespective of the extracellular calcium concentration^{8,9}. In investigating calcium involvement in secretion and the action of ionophores, emphasis has been placed on *in vitro* studies, where the experimental conditions can be closely controlled. We report here the results of experiments on the effect of Br-X537A on the renin levels in *Amphiuma means* kidney in organ culture. This system has the advantages of an isolated tissue, which in addition retains its *in vivo* characteristics for extended periods. We show that Br-X537A has dose-dependent effects on both the synthesis and secretion of renin. These effects apparently represent two quite separate sites of action of the ionophore.

Control of renin secretion is effected through humoral agents, renal sympathetic nerve stimulation, and the intrarenal receptors sensitive to pressure or to ion concentrations¹⁰. Renin release by the kidneys is of fundamental importance in the maintenance of blood pressure and sodium homeostasis. The interpretation of *in vivo* studies of drug action on renin secretion is complicated by the interplay of the control mechanisms^{10–13}. To distinguish between direct and indirect actions of drugs on renin release, isolated perfused kidney¹⁴ and kidney slices^{2,15} have been used *in vitro*. These methods avoid the complications

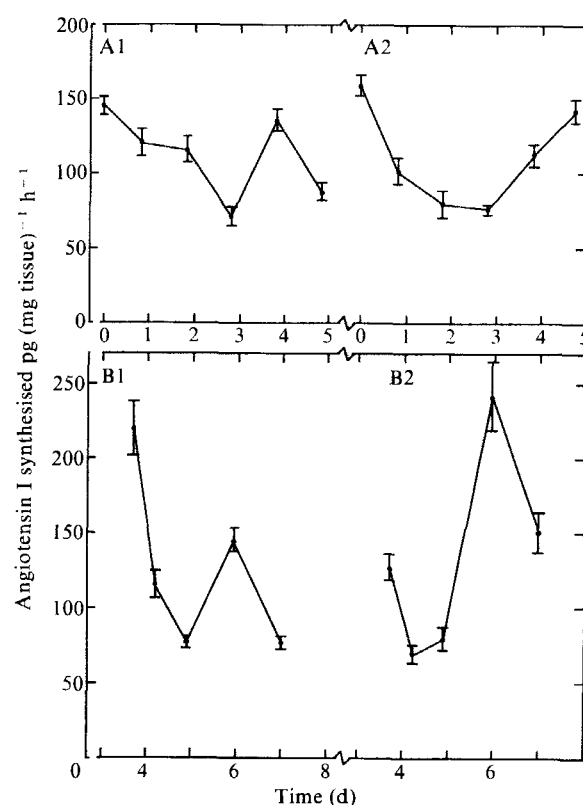


Fig. 1 The renin activity of *A. means* kidney tissue in culture over several days after initiation of culture. Kidneys from two freshly killed animals were immediately removed to culture medium and cut into pieces of average weight 1.5 mg. The pieces were cultured (about 25 mg per 2 ml of medium) in sterile polystyrene universal containers on a roller culture apparatus at 25 °C. Basic culture method was as described by Monnickendam and Balls²⁰. The medium was 50% Eagle's minimum essential medium with 10% foetal calf serum buffered with HEPES (15 mM). Streptomycin sulphate (100 µg ml⁻¹) and benzyl penicillin (100 IU ml⁻¹) were added. Renin activities were measured by radioimmunoassay of angiotensin I generated from exogenous renin substrate, using a method based on that of Cohen *et al.*²¹ and Haber *et al.*²². The renin activities are expressed as the amount of angiotensin I generated per mg of tissue (wet weight). The bars represent s.e.m., and the number of cultures for each point was either 7 or 8, except for day 0 (fresh tissue) where renin activities were measured in tissue equivalent to three cultures. Each trace shows the renin activity in tissue from a single kidney: A1 and A2 referring to the two kidneys from one animal (measurements made from day 0 to day 5); B1 and B2 to kidneys from the second animal (day 3 to day 7).

of haemodynamic and humoral factors. However, it is known that tissue slices from adult mammalian kidneys lose their normal structure and function in quite short periods ranging from 20 s to 3 d, depending on the experimental conditions^{16–19}.

By contrast, many amphibian tissues in organ culture maintain their structure and biochemical functions for periods of up to 5 weeks²⁰. We have shown that *A. means* kidney in culture maintains its renin content for several days (see Fig. 1). This system is therefore useful for studying the long- and short-term effects of drugs without the complications of changes in haemodynamic and sympathetic nervous activity.

As shown in Fig. 1, after an initial drop in tissue renin activity at the start of the culture period, the renin activity fluctuates with time. Cultures from one kidney behave quantitatively in the same way. Tissue from the second kidney of the same animal may show quite different fluctuations. In studying the effect of the ionophore we have, therefore, related renin activities in the tissue and culture medium at given times to those of controls using tissue from the same kidney.

Table 1 Effect of Br-X537A and puromycin on renin activities in *A. means* kidney and culture media

Treatment	Tissue	Renin activity (pg angiotensin I mg tissue ⁻¹ h ⁻¹)	
		Medium	Total
None (control)	160.7 ± 3.0 (9)	9.7 ± 0.9 (9)	170.4 ± 3.4
Br-X537A 2.12 µg ml ⁻¹	357.4 ± 8.2 (8)*	16.9 ± 2.7 (8)*	374.3 ± 9.3*
None (control)	83.9 ± 2.4 (6)	15.0 ± 1.8 (6)	98.9 ± 3.3
Br-X537A 11.5 µg ml ⁻¹	157.0 ± 4.5 (6)*	40.0 ± 1.9 (6)*	197.1 ± 3.3*
Puromycin 0.25 mM	82.8 ± 2.4 (7)	11.6 ± 1.2 (7)	94.4 ± 2.8
None (control)	154.4 ± 12.7 (5)	15.3 ± 1.6 (5)	169.8 ± 14.2
Br-X537A 20.5 µg	129.8 ± 3.8 (5)	34.4 ± 5.0 (5)†	164.2 ± 6.2
None (control)	184.3 ± 2.5 (6)	19.1 ± 1.2 (6)	203.4 ± 2.9
Puromycin 0.25 mM + Br-X537A 11.5 µg ml ⁻¹	160.0 ± 2.8 (6)*	22.0 ± 3.0 (6)	181.7 ± 5.6†

Results are expressed as mean ± s.e.m. with number of cultures in parenthesis. The culture medium and conditions, and method of renin assay were as described in Fig. 1 and the text.

* $P < 0.001$, in comparison between treated and control values in the same experiment.

† $P < 0.01$.

For drug treatments fresh kidney tissue was cultured at 25 °C for 20–24 h. The medium was then changed to either fresh medium (controls) or to fresh medium containing the drugs. After treatment for 24 h the kidney pieces were removed, rapidly blotted, weighed, frozen and kept at –20 °C until assayed for renin activity. Media containing the ionophore were prepared from solutions of the drug in dimethyl sulphoxide. In control cultures dimethyl sulphoxide was added to give the same concentration as in the treated cultures. The concentration of the solvent never exceeded 0.5% v/v. Lactate dehydrogenase (LDH) activity in the medium was measured to check for nonspecific leakage of cell contents. The amount of LDH in the medium was less than 0.5% of that measured in the tissue.

The effects of Br-X537A and puromycin on renin activity are shown in Table 1 and Fig. 2. Over 24 h puromycin alone has no effect. The ionophore gives two dose-dependent effects on renin levels. The first effect is on renin synthesis. There is a stimulation of total (tissue + medium) renin

activity at low ionophore concentrations, and this effect is progressively inhibited as the ionophore concentration is increased. That this is due to enhanced renin synthesis and not to some other action of the ionophore is indicated by the observation that the presence of both puromycin and ionophore (11.5 µg ml⁻¹) together causes only a small decrease in the total renin activity. The second dose-dependent effect is on renin release. In order to distinguish the effect on renin release as distinct from synthesis, we can express the medium renin activity as a fraction of the total renin activity present, f . Then to take into account the variability between different kidneys, f is normalised to the control value (see Fig. 2 insert). Then it is apparent, that puromycin has no significant effect on renin release and, as has been observed by other workers using the neurohypophysis⁸, the amount of hormone released increases with ionophore concentration. However, the validity of interpreting the results quantitatively this way depends on all the tissue renin being equally available for release.

The secretagogue^{4,8}, pseudofertilisation²³, and cardiovascular⁸ actions of X537A and Br-X537A are usually interpreted as being mediated by mobilisation of Ca²⁺ ions and/or catecholamines. We have been unable to show a significant effect of isoprenaline or adrenaline on renin levels in *A. means* kidney *in vitro* over the time scale of the present experiments (data not shown); this suggests that intracellular Ca²⁺ activities are critical in determining renin synthesis, in a manner reminiscent of steroidogenesis in the adrenal cortex²⁴, and synthesis of hormones in the pituitary²⁵. We have been able to demonstrate the two separate actions of Br-X537A on renin levels (synthesis and secretion), because we have used an *in vitro* kidney system that retains its renin for extended periods.

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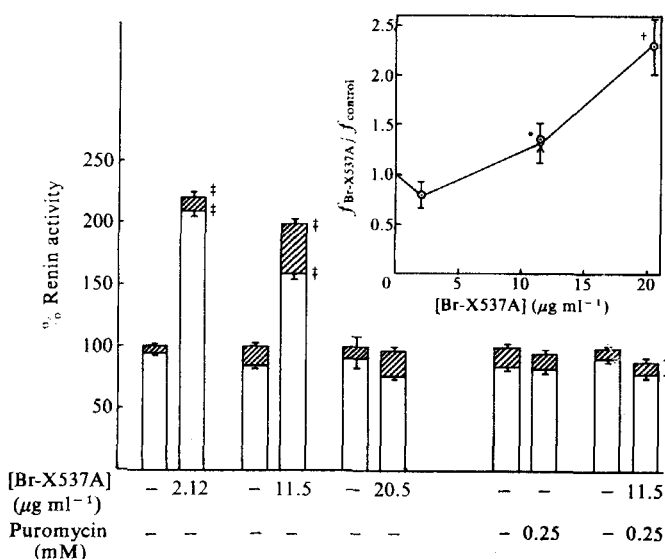
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Fig. 2 To show the different dose dependences of renin synthesis and release, renin activities are shown as a % of the total control values. Open bars represent tissue activity and cross-hatched areas represent medium renin activity. Error bars represent s.e.m. The insert shows $f_{\text{Br-X537A}}/f_{\text{control}}$, (fraction of total renin in medium in the presence of ionophore/fraction of total renin in medium in untreated control cultures) as a function of ionophore concentration. ×, Puromycin present. *, $P < 0.05$, †, $P < 0.01$, ‡ $P < 0.001$, where comparisons are made between cultures with and without drugs. In the insert, the P values refer to comparing $f_{\text{Br-X537A}}$ with f_{control} .



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Angiotensin II regulates its receptor sites in the adrenal glomerulosa zone

DURING sodium deficiency, adrenocortical sensitivity to angiotensin II is increased in several species¹⁻³. Following acute sodium restriction in the rat, adrenal receptor sites for angiotensin II show increased concentration and binding affinity, and aldosterone responses to angiotensin II are increased both *in vivo* and *in vitro*⁴. Since circulating renin and angiotensin II levels are elevated during sodium deficiency^{2,3}, these changes in the adrenal gland could be mediated by the trophic action of angiotensin II on the zona glomerulosa. Increased levels of catecholamines and trophic peptide hormones, such as luteinising hormone, have been shown to cause receptor loss and desensitisation of specific responses in their respective target cells⁵⁻⁷. It is possible, however, that physiological elevations of blood angiotensin II concentration could exert the opposite effect in the adrenal zona glomerulosa, leading to enhanced receptor function and contributing to the increased adrenal sensitivity during sodium restriction. To determine whether angiotensin II modulates its own receptors as well as the aldosterone secretory response, we examined angiotensin II receptors and aldosterone production in the zona glomerulosa of rats following elevation of circulating angiotensin II levels. These studies have provided evidence that angiotensin II can regulate its own receptor sites as well as steroidogenic responsiveness in the adrenal glomerulosa cell.

Isolated zona glomerulosa cells and membrane-rich particles were prepared from adrenal glands of adult rats as described previously⁸. Circulating angiotensin II levels were increased by implanting Alzet osmotic minipumps (Alza Corporation, Palo Alto) intraperitoneally for periods of 36 h-6 d, to give sustained release of synthetic [Asp¹, Ile⁸]-angiotensin II (Beckman Instruments, Inc. Palo Alto), at a rate of 50 ng min⁻¹. The control rats were implanted similarly with empty plastic tubing for equivalent periods. Assay of adrenal receptors by binding of ¹²⁵I-angiotensin II, and measurement of aldosterone in plasma and incubation media, were carried out as described previously^{8,9}.

Plasma aldosterone concentrations were markedly increased from 9.8±2.5 (*n* = 12) to 26.9±5.8 ng per 100 ml (*n* = 13), following infusion of angiotensin II for 36 h-6 d (*P* < 0.001). Angiotensin II infusion was also followed by enhancement of basal and angiotensin-stimulated aldosterone production in isolated glomerulosa cells (Fig. 1). After 36 h of infusion, the maximum aldosterone response *in vitro* was increased from 9.2±0.4 to 26.0±1.3 ng ml⁻¹

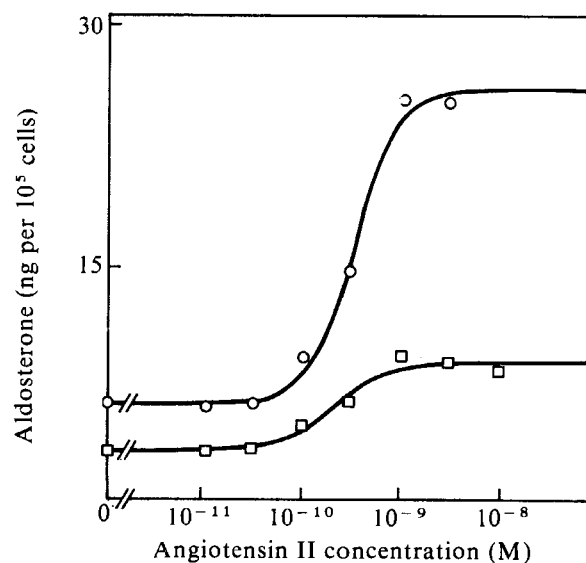


Fig. 1 Stimulation of aldosterone production by angiotensin II in glomerulosa cells from control rats (□) and rats receiving angiotensin II infusion for 36 h (○). Isolated glomerulosa cells were prepared from adrenal capsules of 50 animals in each of the control and angiotensin-infused groups.

(*P* < 0.001). The increased maximum response to angiotensin II *in vitro* was not accompanied by a change in sensitivity to the peptide, as reflected in the angiotensin concentration required to achieve a half-maximum aldosterone response. This rapid and marked change in adrenal responsiveness was accompanied by commensurate changes in the concentration of angiotensin II receptors in the adrenal glomerulosa cell (Fig. 2). A significant increase in the number of angiotensin II receptors was observed after 36 h of angiotensin II infusion, with no change in the association constant of the angiotensin II receptor. Similar increases in the angiotensin II binding capacity (that is, in receptor sites) were observed in adrenal subcellular particles after 36 h (Fig. 3). Such changes were observed for up to 6 d of infusion, whereas receptor affinity was not significantly altered (Table 1).

The rate of angiotensin II infusion used in these studies (50 ng min⁻¹) was accompanied by a slight increase in sodium excretion by the experimental animals. This sodium loss may possibly contribute to changes in angiotensin II receptors and adrenal responses, albeit by an as yet undefined mechanism. The same effects were also observed following infusion of angiotensin II at a rate (20 ng min⁻¹) that had no detectable influence on sodium excretion, however, thus emphasising the ability of the octapeptide to act directly on adrenal receptors and steroidogenic responses.

This study has demonstrated that infusion of angiotensin II causes rapid and significant increases in both angiotensin II receptors and angiotensin-induced responses in the adrenal glomerulosa cells. In contrast, exposure of other target tissues to high hormone concentrations frequently results in reduction of receptor sites and biological effects of the regulatory hormone. Such 'desensitisation' phenomena have been found for luteinising hormone and catecholamines⁵⁻⁷. Other peptide hormones such as insulin, growth hormone, and thyrotropin-releasing hormone decrease their own receptors¹⁰⁻¹², whereas high circulating prolactin concentrations produced by oestrogen treatment are believed to increase the hepatic prolactin receptor¹³. In tissues in which receptors are decreased by elevations of the homologous hormone, an increase in hormone receptors would be expected when the circulating hormone is decreased. Accordingly, both thyrocalcitonin receptors and

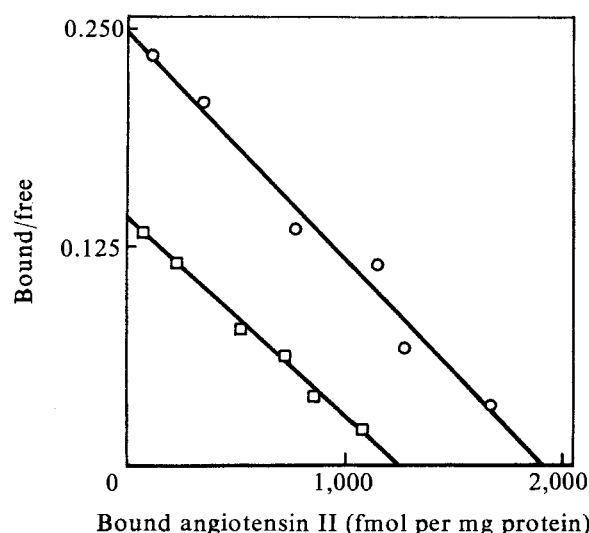
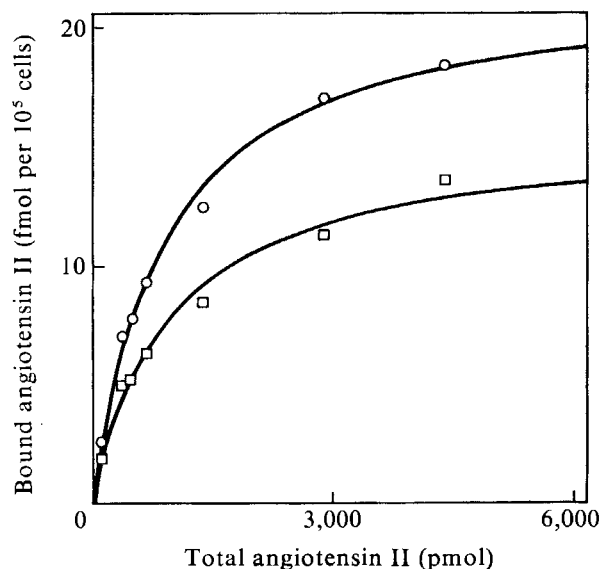
Table 1 Concentration and binding affinity of angiotensin II receptors in adrenal of control and angiotensin II-infused rats

Duration of infusion (d)	Control		After angiotensin II infusion	
	Concentration (fmol mg ⁻¹)	K _a (×10 ⁹ M ⁻¹)	Concentration (fmol mg ⁻¹)	K _a (×10 ⁹ M ⁻¹)
1.5	1,188 ± 40	0.53 ± 0.04	1,766 ± 109	0.56 ± 0.09
3.5	1,697 ± 120	0.84 ± 0.13	2,463 ± 263	0.69 ± 0.12
3.5	1,288 ± 39	1.79 ± 0.03	1,629 ± 74	0.88 ± 0.05
6.5	1,160 ± 52	0.76 ± 0.04	1,911 ± 123	0.61 ± 0.03

Angiotensin II receptor concentrations for all experiments significantly increased ($P < 0.02$) from $1,333 \pm 124$ to $1,943 \pm 183$ (mean ± s.e.) following angiotensin II infusion. Each of the four experiments was performed on adrenal particles prepared from 15 rats in the control and angiotensin-infused groups.

uterine angiotensin II receptors are increased by the absence of the regulatory hormone^{14,15}. The adrenal angiotensin II receptor, however, seems to be regulated in the opposite manner. Thus, angiotensin II receptors in the rat adrenal are reduced after nephrectomy¹⁶ and aldosterone responses of the human zona glomerulosa are reduced following the removal of endogenous angiotensin II by nephrectomy and restored after renal allografts¹⁷. Conversely, sodium restriction, which is associated with increased circulating angiotensin II levels, increases angiotensin II binding sites and the sensitivity and magnitude of the aldosterone response^{2-14,16}.

Administration of pharmacological doses of angiotensin II has been shown to produce a decrease in adrenal receptor sites in nephrectomised rats¹⁸. Such depletion of peptide receptors probably occurs in all target cells after excessive degrees of receptor occupancy, and may bear little relevance to the physiological modulation of target cell functions by normal variations in circulating hormone concentration. In contrast, the administration of nanogram quantities of angiotensin II in the present study was accompanied by a rise in specific adrenal receptors, in keeping with the enhanced aldosterone production seen *in vitro* and *in vivo* after angiotensin infusion. It should be noted that the level of plasma aldosterone produced by angiotensin II infusion was less than that observed during similar periods of sodium deficiency, namely 69.2 ± 7.2 ng per 100 ml after 4 d. This

Fig. 2 Saturation curves of angiotensin II binding data derived with adrenal glomerulosa cells from control rats (□) and rats receiving angiotensin II infusion from 36 h (○). Binding analysis was on the cell preparations used for the aldosterone dose-response studies shown in Fig. 1.**Fig. 3** Scatchard plots of angiotensin II binding in adrenal glomerulosa 30,000g particulate fraction from control rats (□) and rats receiving angiotensin II infusion for 36 h (○). Each set of binding data was derived from adrenal particles prepared from 15 control and 15 angiotensin-infused animals.

difference could reflect the need for a pulsatile or episodic pattern of increased angiotensin II secretion to produce the maximum trophic action on zona glomerulosa cells. The presence of diurnal variation and episodic secretion of renin is well established in man, and pulsatile change may form an important component of the modulating effect of angiotensin II on adrenal glomerulosa function and aldosterone secretion.

We suggest that the direct regulatory effect of angiotensin II on the zona glomerulosa is a major factor in the increased responsiveness of the adrenal cortex during sodium restriction. This view is consistent with earlier studies which showed the administration of renin increases adrenocortical sensitivity to angiotensin II in the dog¹⁹, and with the recent finding that prolonged infusion of angiotensin II in man similarly increases the sensitivity of the adrenal cortex²⁰. Such changes are also accompanied by an increase in ACTH-induced aldosterone responses, indicating a generalised enhancement of the aldosterone biosynthetic pathway^{18,21} that is consistent with a trophic action of angiotensin II on the adrenal gland. In conjunction with these observations, our findings of angiotensin II receptor induction and correspondingly increased steroid responses provide direct evidence that angiotensin II exerts a trophic action on cells of the zona glomerulosa, in addition to its acute regulatory effects on aldosterone secretion.

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Evidence for a long Leu-enkephalin striopallidal pathway in rat brain

THE pentapeptides leucine (Leu-) enkephalin and methionine (Met-) enkephalin were isolated from brain and shown to be endogenous opiates¹. These peptides bind to the brain opiate receptor², and their biological actions are blocked by the morphine antagonist naloxone³. The availability of synthetic enkephalins has led to the development of radioimmunoassays (refs 2, 4, and R. Miller, personal communication) and immunohistochemical techniques. Preliminary immunohistochemical studies^{5,6} have shown the distribution of enkephalin-containing fibres to coincide with the regional distribution of opiate receptors as determined by biochemical techniques and autoradiography^{6–8}. The highest density of enkephalin-positive fibres^{9,8} was located in the globus pallidus. We have now confirmed the presence of enkephalin-immunoreactive fibres in the globus pallidus, and using specific lesions have shown this immunoreactivity to be abolished by destruction of the neural connections between the globus pallidus and the caudoputamen.

The visualisation of the Leu-enkephalin-immunoreactive fibres in the brain tissue was carried out using the indirect fluorescent immunohistochemical techniques first described by Coons and Kaplan⁹ with a Leu-enkephalin antiserum from rabbit and anti-rabbit IgG fluorescein conjugated immunoglobulin (Wellcome). Rat brains were fixed by intracardiac perfusion with 4% cold paraformaldehyde in 0.1 M buffer phosphate and processed as described by Hökfelt *et al.*¹⁰. Cryostat sections 10-µm thick were used for immunocytochemistry. The exact location and extent of each lesion was determined from Nissl-stained sections. Leu-enkephalin antiserum was raised in the rabbit by Dr R. Miller of Burroughs Wellcome. The Leu-enkephalin antiserum did not cross-react with β -lipotropin or adrenocorticotrophic hormone (ACTH), and the cross-reactivity with Met-enkephalin was 1% and with β -endorphin < 0.01%. The specific immunofluorescence in the globus pallidus and other regions was not observed if the Leu-enkephalin antiserum was previously absorbed with Leu-enkephalin (200 µg ml⁻¹). Light microscopy at high magnification showed the immunoreactivity in the globus pallidus to form a dense mesh of intensely fluorescent elements. The lack of immunoreactive elements in the caudoputamen and the abundance in the globus pallidus causes a sharp delineation of the border between caudoputamen and the globus pallidus.

Isolation of the globus pallidus from the neighbouring caudoputamen by a knife-cut lesion within the caudoputamen (Fig. 1, lesion 1) caused an almost complete depletion of Leu-enkephalin immunofluorescence from the ipsilateral globus pallidus (Fig. 2a, b). In 10 animals, the magnitude of this depletion was related to the extent of the lesion in the caudoputamen determined from alternate Nissl-stained sections. Knife cuts limited to the rostral pole of the caudoputamen (Fig. 1, lesion 2) produced a depletion of enkephalin immunoreactivity restricted to the medial and rostral portions of the globus pallidus. This observation suggests a possible topographical arrangement of the enkephalin-containing fibres in the caudoputamen-globus pallidus pathway.

The view that the Leu-enkephalin might be present in the processes of striatal neurones was reinforced by the appearance

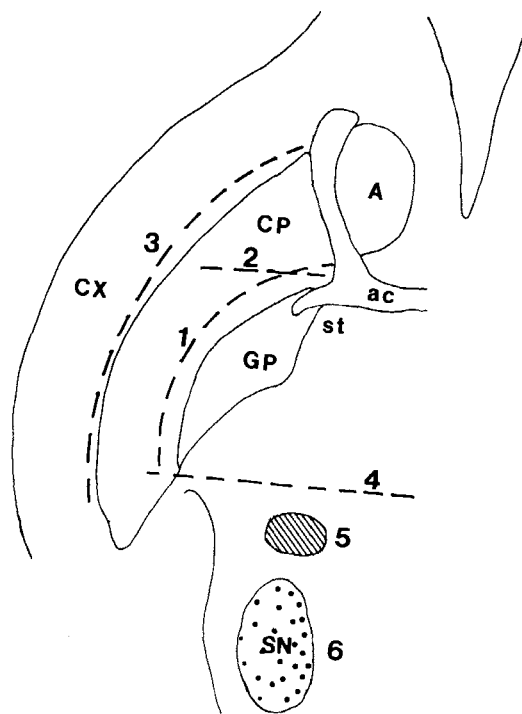


Fig. 1 Schematic representation of a horizontal section of the rat brain. CX, cortex; CP, caudoputamen; GP, globus pallidus; A, nucleus accumbens; ac, anterior commissure; st, nucleus interstitialis of the stria terminalis; SN, substantia nigra. 1, Knife cut isolating the caudoputamen from globus pallidus; 2, knife cut in the rostral pole of the caudoputamen; 3, cerebral cortex knife undercut; 4, brain hemisection; 5, electrolytic lesion of the medial forebrain bundle; 6, intranigral injection of 6-hydroxydopamine. Lesions 1–4 were performed with a stereotactically-guided microknife. Survival time for all procedures was 5–8 d.

of bundles of fluorescent beaded fibres on the striatal side of the knife cuts (Fig. 3). These fluorescent fibres were only seen on the lesioned side, running radially through the striatum towards the globus pallidus. When consecutive sections were incubated with non-immune serum or anti-enkephalin serum pre-absorbed with Leu-enkephalin (200 µg ml⁻¹) the accumulation of fluorescent material proximal to the lesion was not observed. The build-up of enkephalin immunofluorescence observed here is similar to that seen with substance P after dorsal root lesions¹⁰ or knife cuts in the central nervous system¹¹.

Stereotaxic undercutting of the cerebral cortex with a microknife (Fig. 1, lesion 3) did not produce any noticeable change in the intensity or pattern of the pallidal immunofluorescence compared with the contralateral untreated side. Stereotaxic injections of 6-hydroxydopamine (8 µg in 2 µl given over 4 min) into the substantia nigra which in parallel experiments resulted in a 60–70% fall in striatal dopamine content, electrolytic lesions of the medial forebrain bundle (2 mA, 20 s), and stereotaxic knife-cut hemisections at the middle-hypothalamic level, all failed to change the pattern of Leu-enkephalin immunoreactivity in the globus pallidus (Fig. 1, lesions 4–6).

These findings are in agreement with recent biochemical observations which show that the radioimmunoassayable enkephalin content of the basal ganglia does not change after cortical ablation¹⁴, but decreases by 50% following cell body destruction induced by intrastriatal injection of kainic acid⁴. They are also in agreement with the reported appearance of enkephalin immunoreactive cell bodies in the caudoputamen following inhibition of axonal transport by colchicine¹⁵.

Our results suggest that the Leu-enkephalin-containing nerve fibres in the pallidum do not belong to an intrinsic neuronal circuit but instead to a striopallidal pathway. Such a connection between the two basal ganglia nuclei is consistent with classical

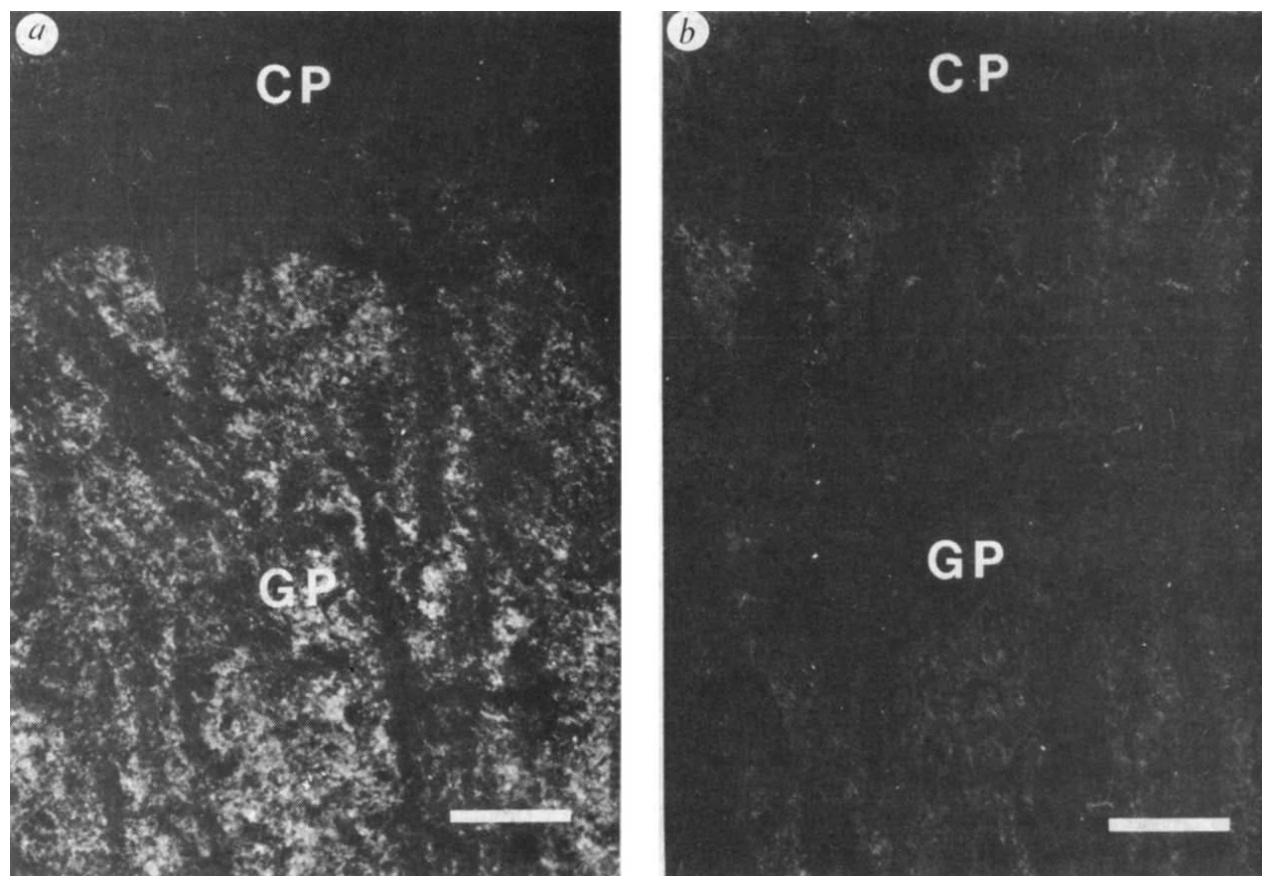


Fig. 2 *a*, Leu-enkephalin immunofluorescence in the globus pallidus (GP). Note the lack of immunoreactivity in caudoputamen. Horizontal section, untreated side. *b*, Ablation of Leu-enkephalin immunofluorescence in the globus pallidus following de-afferentation from the caudoputamen (contralateral to *a*). Scale bar, 225 μ m.

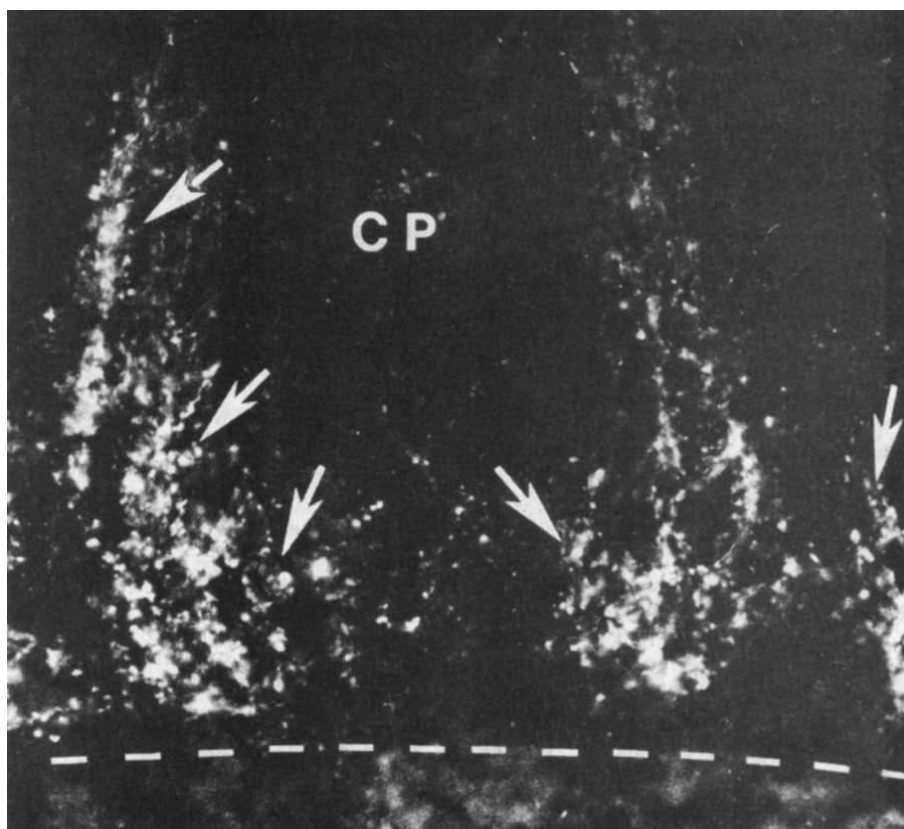


Fig. 3 Build-up (arrows) of Leu-enkephalin immunoreactivity in fibre bundles located in the caudoputamen (CP) at the level of the knife cut (dashed line). Scale bar, 40 μ m.

neuroanatomical studies which show neurones in the caudoputamen to branch extensively within the globus pallidus^{12,13}. The role of this 'long enkephalinergic' striopallidal pathway in extrapyramidal function and disease, remain to be established.

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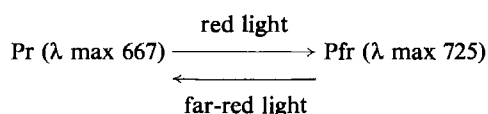
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Evidence for symmetry in the phytochrome subunit

PHYTOCHROME is a chromoprotein that has been shown to initiate and control major developmental responses of plants to light¹. It has two spectrally different forms that can be reversibly interconverted by light, as indicated:



The spectral properties of phytochrome are sensitive to changes in the microenvironment of the covalently bound chromophore(s). Relatively mild denaturing treatments, such as incubation at 25 °C for over an hour² and dehydration³, result in a significant loss of photoreversibility. In view of this susceptibility to spectral denaturation, it is remarkable that phytochrome can be proteolytically degraded to a molecular weight (MW) of 60,000, or half its native subunit size, without exhibiting any loss of photoreversibility or even any blue shift in its peak absorbance as Pr⁴. The additional fact that further proteolysis of phytochrome to 40,000 MW drastically reduces photoreversibility⁵ suggests that the 60,000 MW fragment has a sequence and configuration that are both sufficient and necessary to preserve photoreversibility. It is not clear whether this fragment is the product of one or more centrally located

cleavages of the native subunit to yield two pieces of MW near 60,000 per native subunit or whether it is the product of extensive proteolysis which yields a single 60,000 MW fragment per native subunit. We have compared the amino acid composition and the peptide map of the proteolytically-derived 60,000 MW fragment with those of the native 120,000 MW subunit. Our results strongly support the conclusion that the native 120,000 MW subunit is composed of two halves with nearly identical sequences.

To assure maximum purity, phytochrome was isolated from single bands on sodium dodecyl sulphate (SDS) gels after the preparative electrophoresis method of Stephens⁶. The samples used for the preparative electrophoresis were 70-80% pure and were isolated from etiolated oats by the method of Roux *et al.*⁷. By varying the extraction conditions this method can be used to produce both native phytochrome with intact 120,000 MW subunits and also the 60,000 MW fragment of phytochrome⁷. The MW of the smaller fragment was confirmed to be 60,000 by chromatography on a calibrated molecular sieve gel before electrophoresis on SDS gels. After the preparative electrophoresis, the purity and molecular weight of the samples were confirmed by re-electrophoresing aliquots of them on SDS gels. In all cases, 10 µg samples gave single bands at either 60,000 or 120,000 MW, indicating over 95% purity.

Table 1 compares the amino acid composition of the pure 60,000 and 120,000 MW phytochrome. This is the first report of the amino acid composition of the native phytochrome subunit. One analysis of each molecular weight species is shown. A second set of analyses on different phytochrome preparations that were also purified by the electrophoresis method gave identical results. To show that the preparative electrophoresis method of isolation does not produce composition artefacts and to further indicate the reproducibility of the results, the composition of 60,000 MW phytochrome isolated by standard chromatographic methods⁸ is given alongside of the electrophoretically isolated phytochrome.

The data in Table 1 reflect the composition values in mol % after subtracting the background values of a blank. This background contamination level was determined by amino acid analysis of material extracted from blank areas of the preparative gels. The reproducible contamination level was low but measurable and typical of that reported by others using the same technique⁹. The standard error limits for the final extrapolated values given are ± 0.2 mol % for all residues except Gly and Asp, where the error limits are about ± 1.0 and ± 0.5 mol % respectively. Thus the 120,000 and 60,000 MW peptides have statistically the same compositions at six residues (Lys, His, Arg, Ser, Met, and Ile). The differences at the other residues range from barely significant (Cys, Phe, Thr: 0.3 mol %) to 1.6 mol % for Gly.

The composition data are consistent with the hypothesis that the isolated 60,000 MW fragments were a heterogeneous mixture of two dissimilar halves of the native subunit. But they also suggest the possibility that the subunit may be composed of two nearly identical 60,000 MW regions. To distinguish which of these conclusions was more likely, tryptic peptide maps of the two phytochrome species were prepared. If the native subunit had little or no internal symmetry, then, ideally, one would expect to identify a number of tryptic peptides equal to the number of Lys and Arg residues in the native subunit plus one. On the other hand, the greater the symmetry, the fewer the number of different peptides that would separate on the map, with the lower limit being the number of peptides that were separable on the map of the 60,000 MW fragment. From the composition data, there were 59 lysines and 49 arginines per 120,000 MW subunit and 30 Lys and 24 Arg in the 60,000 MW fragment.

Complete tryptic digests of electrophoretically pure 120,000 and 60,000 MW phytochrome were prepared and chromatographed as described in the legend to Fig. 1. Two maps of two separately purified samples were made for both the intact

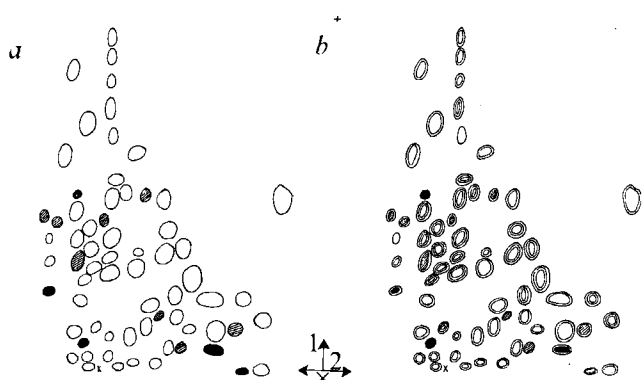


Fig. 1 *a*, Peptide mapping on thin-layer Silica G plates was patterned after a procedure by Stephens¹⁶. The isolated protein was subjected to complete tryptic digestion using a protein to DCC-trypsin ratio of 100:1 (w/w) for 24 h at 25 °C in 1% (NH₄)₂CO₃. The reaction was terminated by addition of several drops of glacial acetic acid followed by lyophilisation with three washes of distilled water. The digests were chromatographed on 20 × 20 cm Silica G plates in butanol-acetic acid-water (80:20:20) and air-dried overnight. High voltage electrophoresis in a perpendicular direction was performed on a Savant flat bed high voltage electrophoresis unit at 1,000 V for 105 min using pyridine-acetic acid-water (100:8:397), pH 6.5, solvent. The plates were oven-dried for 1 h at 110 °C. The final map was visualised with ultraviolet light after spraying with fluorescamine (0.025% in acetone). The fluorescing map was photographically documented using Kodak contrast process pan film. This figure represents a composite of six different peptide maps; three of the 120,000 MW subunit and three of the 60,000 MW fragment. The open circles denote those peptides common to both phytochrome species (55). Shaded circles denote peptides seen only on maps of the 120,000 MW subunit. Cross-hatched circles denote peptides only on maps of the 60,000 MW fragment. *b*, This is the same composite as (*a*) but the intensity of fluorescence is recorded. Three concentric circles denotes bright, two concentric circles denotes moderate, and a single circle denotes dim intensity.

subunit and its fragment. The peptide 'fingerprints' were visualised after fluorescamine treatment by their fluorescence under ultraviolet light. From photographic prints of the maps, *x-y* coordinates and relative intensity values were assigned to each peptide spot, and from these assignments the composite map shown in Fig. 1 was drawn.

The composite map illustrates that we were able to identify only 60 different peptides on the map of the 120,000 MW subunit, and of these 55 were in the same relative position and had

the same relative intensity as distinct peptide spots on the map of 60,000 MW phytochrome. As shown in Fig. 1, we identified 63 peptides on the map of the 60,000 dalton fragment, eight more than the ideal maximum predicted from the Lys and Arg content of the fragment. This anomaly can probably be ascribed to the likely size microheterogeneity of the 60,000 MW preparation. Such heterogeneity would be expected as this fragment is produced by the action of a variety of endogenous oat proteases¹⁰, none of which, presumably, are trypsin. Direct evidence of the heterogeneity of 60,000 MW phytochrome was provided by Rice and Briggs¹¹, who identified four different N-terminal amino acids in a preparation which was homogeneous in SDS gel analysis. The fact that two of the peptides unique to the 60,000 MW fragment only dimly fluoresce suggests that they are minor components of the population and further supports the hypothesis of microheterogeneity within the population of these fragments.

To rule out the possibility that some of the brighter spots represented multiple peptides whose fluorescent intensity masked their resolution as separate spots, we ran additional maps on reduced quantities of tryptic digests. Though these maps gave greater resolution of the brighter spots, they did not reveal any new spots. To demonstrate that our chromatography conditions would allow the separation of more spots, if they were present, we ran maps of phytochrome samples which were only 80% pure. We were able to identify up to 12 new spots on these maps, in addition to those depicted in Fig. 1.

The data presented above strongly support the hypothesis that the native subunit of phytochrome has extensive regions of internal symmetry in its primary structure. Because the compared amino acid compositions and peptide maps are not identical, it is unlikely that the symmetry is complete. These data do not show whether the symmetry is tandem or bilateral, nor do they reveal whether the symmetry extends to the number and position of the chromophore(s) of phytochrome. Our results contradict the conclusion inferred from immunochemical evidence that the 60,000 MW fragment of phytochrome bears little resemblance to the other half of the native subunit¹³. But these data do not argue against the conclusion that the native subunit is, in fact, of MW 120,000. The evidence for the latter¹¹ seems incontrovertible.

It is not known whether the extensive sequence duplication in phytochrome has any functional significance. Information on this point may come from experiments in progress to compare the competence of 60,000 and 120,000 MW phytochrome to affect the properties of native¹³ and model¹⁴ membranes.

Table 1 Amino acid composition of 120,000 and 60,000 MW phytochrome

	Residues in mole %			Comparative data based on available 24-h hydrolysates†	
	Complete composition* 120,000	Complete composition* 60,000	60,000	60,000‡	60,000§
Cysteic acid	1.5	1.8	—	ND	ND
Aspartic acid	10.4	9.6	9.7	10.0	10.0
Threonine	4.2	4.5	4.3	4.3	4.4
Serine	7.3	7.5	6.8	6.8	6.5
Glutamic acid	11.4	10.2	10.9	10.4	10.0
Proline	4.6	5.6	6.0	5.6	6.1
Glycine	5.9	7.5	7.8	6.9	6.6
Alanine	8.8	9.3	9.4	9.6	9.7
Valine	7.4	6.7	6.6	7.1	6.7
Methionine	2.8	2.8	2.7	2.7	2.7
Isoleucine	4.9	4.7	4.8	5.1	4.8
Leucine	11.2	10.1	10.2	10.4	11.1
Tyrosine	2.2	2.6	2.5	2.6	2.8
Phenylalanine	4.1	4.4	4.5	4.5	4.6
Lysine	5.6	5.5	5.9	6.1	6.3
Histidine	3.0	2.8	3.1	3.0	3.1
Arginine	4.7	4.5	4.8	4.9	4.6

ND, not determined.

* Combined data from 24, 48, 72 h hydrolysates. Thr and Ser determined by linear extrapolation to zero time. Cys determined as cysteic acid after hydrolysis in the presence of dimethylsulphoxide according to Spencer and Wold.¹⁵

† Data compared only for phytochrome from *Avena sativa*, cv. Garry.

‡ Data from ref. 8.

§ Data from work of Fry and Mumford (see ref. 8).

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Neutron diffraction studies on selectively deuterated phospholipid bilayers

NEUTRON diffraction combined with the use of selectively deuterated lipids can provide detailed information on the molecular structure of membranes. Because of the large difference between the coherent scattering length of hydrogen (-3.74 fermis) and deuterium (6.67 fermis) the deuterated membrane segments show up as intense peaks in the neutron density profile and can thus easily be located in the membrane^{1–3}. We have applied this method to bilayer membranes of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), selectively deuterated at 12 different positions in the polar head group and the hydrocarbon chains. We report here that the mean position of the deuterated segments within the membrane can be determined in most cases to a precision of better than ± 1 Å. The average orientation of the phosphocholine group in the gel state as well as in the liquid crystalline state is almost parallel to the membrane surface. In the gel state the two hydrocarbon chains are out of step by about 1.8 Å, and water penetrates up to the glycerol backbone of the lipid molecules.

The selectively deuterated DPPC molecules were synthesised as described elsewhere^{4,5}. The measurements were carried out at low-water content (5–6 weight % water; 20 °C) and at high-water content (25 wt % water; 28 °C, 38 °C, and 50 °C). At low-water content the multilayered system was orientated on quartz slides and the Bragg reflections were measured in so-called $\theta - 2\theta$ scans¹ on the D16 diffractometer at Institut Laue-Langevin (ILL). The mosaic spread measured for these samples had a half width at half height of about 12°. Figure 1a, b shows the first 10 lamellar orders for DPPC deuterated in both chains at the fifth carbon atom for the first sample and at the 15th carbon atom for the second. Only the first orders are very strong whereas the higher orders are so weak that most of them can hardly be seen in this plot. The quite dramatic differences in the ratios of the peaks on changing the label position indicate the sensitivity of the method. For the high water content samples, the lipid mixed with 25% water was sealed into cells with quartz windows. The resulting diffraction pattern consists of Debye-Scherrer rings which were observed with a two-dimensional position sensitive detector⁶ (the D11 camera at ILL).

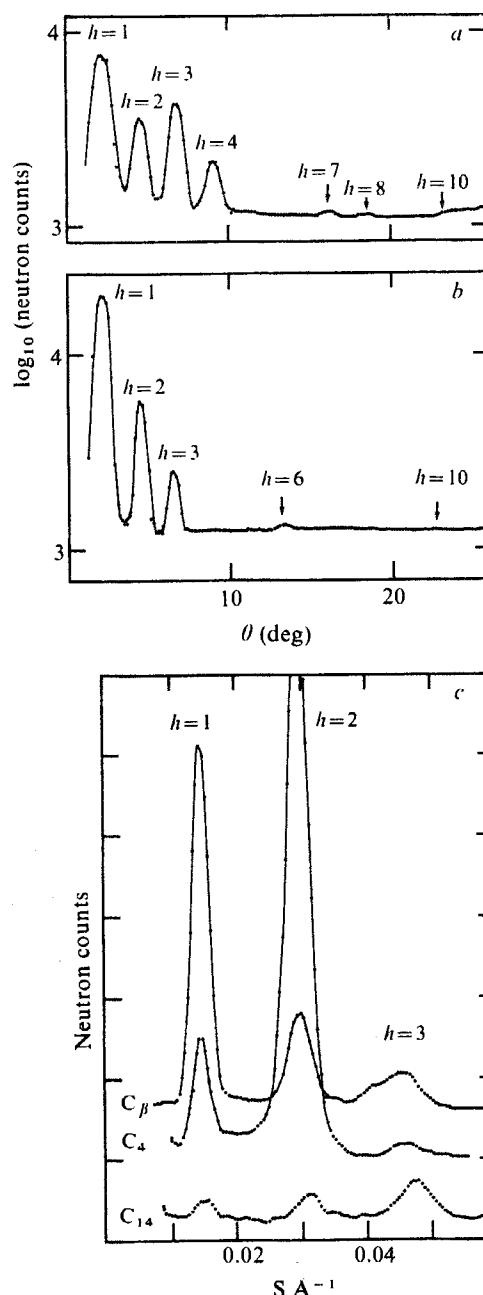


Fig. 1 Diffraction pattern of the lamellar reflections of DPPC multilayers. *a, b*, 10 orders from a $\theta - 2\theta$ scan of two DPPC samples deuterated at the C15 (*a*) and C5 (*b*) position respectively. Each sample was orientated on quartz slides (water content 5–6% wt H₂O, 20 °C, $d = 57.4$ Å, $\lambda = 4.43$ Å, θ = Bragg angle). *c*, Profiles of three Debye-Scherrer rings of powder samples deuterated at C _{β} , C4 and C14 position respectively, as measured on D11 at ILL (water content 25% wt H₂O, 28 °C, $d = 62.5$ Å, $\lambda = 6.26$ Å, $s = 2 \sin\theta/\lambda$). In order not to complicate the picture the three curves were shifted with respect to each other.

Because of inherent disorder in these samples the intensities of the diffraction orders become quite rapidly smaller with increasing scattering angle and it was difficult to observe more than four reflections. The first three orders, shown in Fig. 1c, illustrate the large changes in the ratios of the reflections when different segments of the lipid are deuterated. From these intensities, after appropriate corrections, the structure factors have been derived. Since the structure is centrosymmetric the phase problem is reduced to the choice between 0 and π . Compared to X-ray analysis, neutron diffraction offers several advantages for phasing and also for scaling the individual samples. Thus all the strong

orders are phased by using the isomorphous replacement technique where H₂O in the bilayer is successively exchanged against D₂O (refs 1-3).

Figure 2a and b contains the density profiles of DPPC/H₂O mixtures labelled at the C15 and C5 positions and correspond to the diffraction patterns of Fig. 1a, b. Figure 2c is the difference profile of the C5-labelled lipid in D₂O and in H₂O showing the water distribution.

In principle, it is possible to obtain the position of the deuterium label directly from the density profiles: however, this procedure introduces some uncertainties because of Fourier series truncation errors. In addition, it would have been very time consuming to determine higher orders precisely for all the samples in different conditions (H₂O-D₂O-water content, temperature) as in most cases these orders are weak. Therefore we restrict ourselves to measuring only the strong reflections and to calculating the label positions in reciprocal space by the following procedure. After scaling and phasing each set of structure factors, the appropriate differences between pairs of different sets were derived. These difference sets were then used in a model fitting calculation in which the label distribution was assumed to be gaussian. Mean positions for the labels were therefore obtained with all 12 data sets contributing in each case.

Table 1 summarises the mean positions for the various deuterated segments of DPPC bilayers; the DPPC structure is given in Fig. 3. The distances are measured from the centre of the bilayer. The thermodynamic state of the bilayer is designated according to Luzzati and Tardieu^{7,8}. At low-water content (5-6 wt %) and 20 °C the bilayer is in the gel state and from the separation of the C2 segments of the fatty acyl chains the thickness of the hydrocarbon region is found to be 37.6 ± 2 Å. This result represents an average value, however, since it has been obtained from an experiment in which both fatty acyl chains were deuterated. Labelling the two chains individually reveals different distances for the two C2 segments. The C2(2) segment of fatty acyl chain 2 (attached at the C2 atom of the glycerol backbone) is about 1.9 Å further away from the centre of the bilayer than the corresponding C2(1) segment in the adjacent fatty acyl chain (attached at the C1 atom of the glycerol backbone). Furthermore, model calculations on the density profile of C15-labelled DPPC (Fig. 2a) also show that the two deuterated C15 segments of the same lipid molecule are separated in the bilayer by about 1.8 Å. These results then prove that the two hydrocarbon chains of DPPC in the membrane are out of step by about 1.5 carbon-carbon bonds with fatty acyl chain 2 shifted closer to the membrane surface. A similar finding has been reported for single crystals of dilauroyl-phosphatidylethanolamine⁹. On the other hand, the three segments of the choline moiety (C_α, C_β, C_γ) are positioned at almost

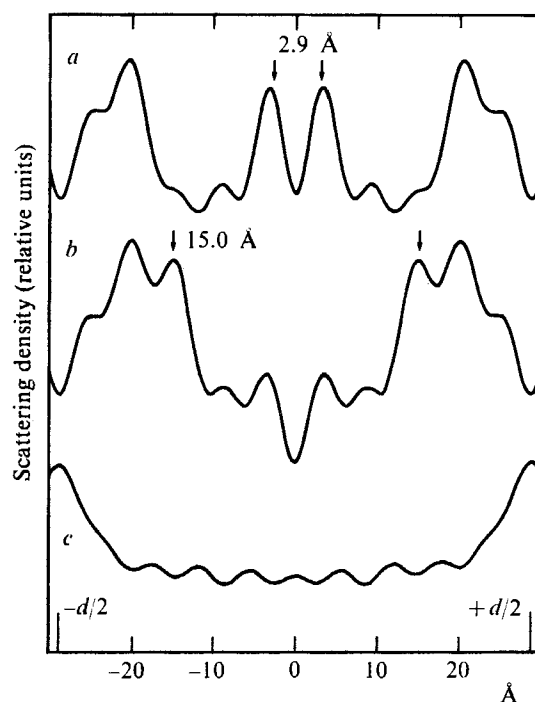


Fig. 2 6-Å resolution scattering length density profiles of DPPC at low-water content (5-6%) and 20 °C. a, deuterated at the C15 position; b, at the C5 position; c, water distribution determined from a difference profile of C5 in D₂O and H₂O. The lamellar spacing was 57.4 Å.

the same distance from the centre of the bilayer, which proves unambiguously that the phosphocholine dipole is orientated parallel to the membrane surface. This is further substantiated by comparing the experimental results with corresponding distances measured from a space-filling molecular model of DPPC (column 3). In this model the two fatty acyl chains were assumed to be out of step by 1.8 Å. Furthermore, in order to comply with the X-ray measurements⁸ both chains were tilted by 16° with respect to the bilayer normal. The choline head groups were either aligned parallel (column 3a) or extended perpendicular (column 3b) to the membrane surface. Table 1 shows that only the parallel head group orientation is in agreement with the experimental findings.

The last three columns represent the results at high-water content. In the L_β phase an increase in the water content from 5 wt % to 25 wt % changes the tilt angle of the hydrocarbon chains

Table 1 Summary of the mean label positions of DPPC in the membrane

	5-6 wt % water 20 °C L _β phase <i>d</i> = 57.4 Å	Distances in a space-filling model		28 °C L _β phase <i>d</i> = 62.5 Å	25 wt % water* 38 °C P _β phase <i>d</i> = 62.2 Å	50 °C L _α phase <i>d</i> = 54.1 Å
		head surface <i>a</i>	head⊥surface <i>b</i>			
Labels in the head group						
C _γ	25.1 ± 0.6	24.4	29.6	24.4 ± 0.6	24.5 ± 0.6	21.8 ± 0.6
C _β	24.8 ± 0.7	25.3	28.0	24.1 ± 1	23.9 ± 1	21.2 ± 1
C _α	24.5 ± 0.7	24.0	27.2	23.6 ± 1	23.0 ± 1	21.0 ± 1
GC3	23.1 ± 1.0		23.5	21.6 ± 1.5	21.4 ± 1.5	17.4 ± 1.5
Labels in the chains						
C2(2)	20.0 ± 1.0		19.4			
C2	18.8 ± 1.0		18.5			
C2(1)	18.1 ± 1.0		17.6			
C4	16.2 ± 0.6		16.1	15.4 ± 1.5	15.3 ± 1.5	12.2 ± 1.5
C5	15.0 ± 0.6		14.9	13.7 ± 1.5	12.2 ± 1.5	10.5 ± 1.5
C9	10.1 ± 1.0		10.1	9.8 ± 1	9.4 ± 1	8.1 ± 1
C14	4.1 ± 0.6		4.1	4.1 ± 1	3.6 ± 1	3.6 ± 1
C15	2.9 ± 0.6		2.9	2.5 ± 1	2.0 ± 1	1.9 ± 1

See Fig. 3 for structure of DPPC showing label positions.

*The three bilayer phases were also investigated by means of X-ray diffraction. The various patterns agree with those published by Janiak *et al.*¹⁰.

from 16° to 30° (ref. 10), leading to a concomitant reduction of the projected length of the hydrocarbon chains by $1.75 \text{ \AA}$. This is also reflected in the position of the deuterium labels which in the high-water content L_β phase are shifted closer to the bilayer interior (column 4). At the pre-transition temperature of 35°C the bilayer structure changes from L_β to P_β . According to Janiak *et al.*¹⁰ the P_β phase is characterised by a two-dimensional lattice with a rippled bilayer. The label positions as seen by neutron diffraction are almost the same in the L_β and P_β phase (column 5), however, and we therefore conclude that this effect produces only minor structural changes in the projection normal to the bilayer plane. It could be argued that the observed intensities of the $I(h, 0)$ rings also contain contributions from the weak $I(h, k)$ reflections which

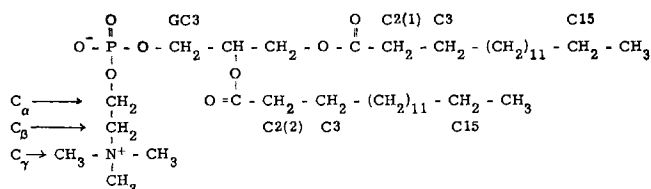


Fig. 3 Structure of DPPC molecule showing labelling positions (see Table 1).

would reduce the accuracy of our structure factors. Analysing the densitometer tracing of the P_β phase X-ray picture we can estimate a maximum error of 6% in the structure factors if three orders of $I(h, k)$ are superimposed on one $I(h, 0)$ reflection. This error does not seriously affect the determination of the mean label positions.

At 50°C the DPPC bilayers are in the liquid crystalline state with disordered hydrocarbon chains. This is evidenced by a remarkable contraction of the DPPC bilayer by about $6\text{--}8 \text{ \AA}$, but again the three segments of the choline moiety exhibit almost the same distance from the centre of the bilayer. Thus regardless of whether the membrane is in the gel or in the liquid crystalline state and independent of the water content the phosphocholine dipole is always parallel to the plane of the membrane. We are at present investigating the effects of ions on the head group structure.

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Proximity of two tryptophan residues in dihydrofolate reductase determined by ^{19}F NMR

NUCLEAR magnetic resonance methods have provided a wealth of information on the conformational and ligand-binding properties of proteins^{1–3}. In such studies, it is necessary to resolve resonances from the ligand or from individual residues of the protein. To achieve this, it is often necessary to resort to isotopic substitution methods such as selective deuteration^{4,5} or ^{13}C enrichment^{6–8}. An alternative approach is to introduce a fluorine atom (^{19}F) into the system as an NMR probe, either by using fluorine-containing ligands^{9–11} or by labelling the protein itself. This can be done either by chemical modification^{12–15} or by the biosynthetic incorporation of selected fluorinated amino acids^{16–19}. We have used this latter approach to prepare *Lactobacillus casei* dihydrofolate reductase containing either 3-fluorotyrosine or 6-fluorotryptophan residues; by measuring the ^{19}F spectra at 94.1 MHz we have characterised the effects of ligand binding on individual tyrosine and tryptophan residues in the protein¹⁹. We now report through-space ^{19}F – ^{19}F spin–spin coupling in 6-fluorotryptophan containing dihydrofolate reductase which demonstrates that two of the tryptophan residues are in close proximity in the folded structure of the protein.

Figure 1a shows the ^1H noise-decoupled ^{19}F NMR spectrum at 94.1 MHz of 6-fluorotryptophan-containing dihydrofolate reductase in its ternary complex with methotrexate and NADPH. Each of the five fluorotryptophan

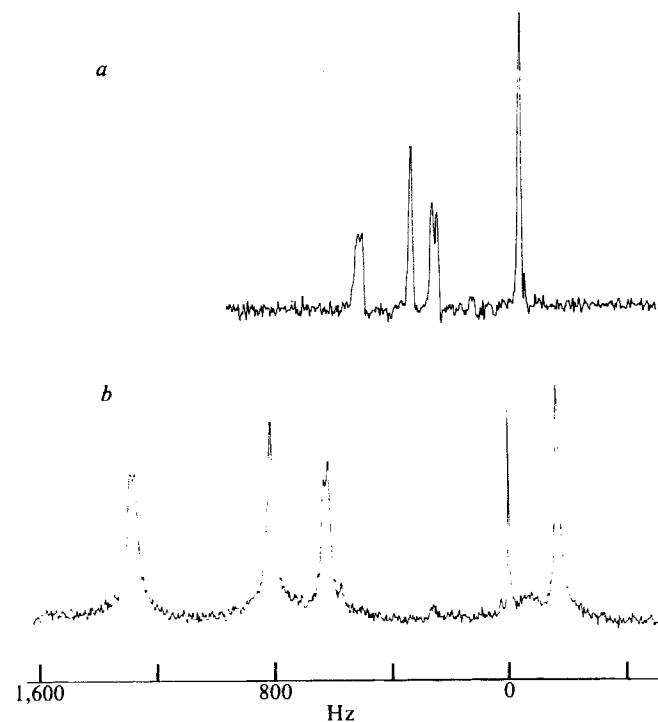


Fig. 1 a, The proton noise-decoupled ^{19}F NMR spectrum at 94.1 MHz of a 1 mM solution of 6-fluorotryptophan-labelled dihydrofolate reductase in the presence of 1 equivalent of methotrexate and 1 equivalent of NADPH. The sample was examined as a D_2O solution containing 50 mM potassium phosphate, 500 mM potassium chloride, $\text{pH } 6.5$ (meter reading); sample temperature was 11°C . Details of the purification of the enzyme and experimental conditions used for recording the spectrum are given in ref. 19. The high field signal has been assigned to two overlapped ^{19}F signals; however, more recent sequence studies (K. Batley and H. R. Morris, personal communication) suggest that the enzyme contains only four Trp residues. b, The proton noise-decoupled ^{19}F NMR spectrum of the same sample at 254 MHz (sample temperature 28°C). The additional sharp signal at zero frequency arises from a small amount of denatured enzyme which appears on standing.

residues gives rise to a ^{19}F NMR signal. It can be seen that two of the signals appear as doublets, with components of approximately equal intensity and with splittings of equal magnitude (17 ± 2 Hz). Clearly, since all the ^1H nuclei are being irradiated, these splittings cannot arise from ^1H - ^{19}F spin-spin interactions. The two remaining possibilities are, first, that the splitting is a spin-spin coupling, which must be a ^{19}F - ^{19}F coupling since there are no other spin- $\frac{1}{2}$ nuclei present a 100% abundance in the system or, second, that the splitting is a chemical shift difference, reflecting the presence of two equally populated conformational states of the protein. These two possibilities can be distinguished either by spin-decoupling ($\{^{19}\text{F}\}$ - ^{19}F) or by studying the field-dependence of the splitting, since chemical shift differences are directly proportional to the strength of the applied magnetic field, while spin-spin couplings are field independent. We have now examined the ^{19}F spectra at 2.7 times higher field strength (254 MHz for ^{19}F) and find that the splittings are again 17 ± 2 Hz, that is they are independent of the operating frequency and thus correspond to spin-spin interactions and not to chemical shift differences. The constancy of the splittings can be clearly seen in Fig 1a and 1b where the spectra at 94.1 and 254 MHz are presented on the same frequency scale. Instrumental limitations have prevented us from carrying out the triple resonance experiment (^1H and ^{19}F irradiation) required to demonstrate ^{19}F - ^{19}F homodecoupling. However, we have been able to confirm that the doublets arise from homonuclear ^{19}F - ^{19}F spin-coupling by studying the ^{19}F spectra obtained using Carr-Purcell pulse sequences ($\pi/2-\tau-\pi-\tau$)²⁰. In these experiments the singlet echo resonances decayed as a function of $\exp(-2\tau/T_2)$ whereas the doublet echoes decayed as $\exp(-2\tau/T_2) \cos(2\pi J\tau)$ as would be expected for doublets arising from homonuclear spin coupling. When $2\tau=1/J$, inverted signals are observed for such a doublet while the singlets are not inverted. An analysis of the amplitude of the high field doublet signal (see Fig. 1) as a function of τ gives $T_2 = 38 \pm 8$ ms and $J_{\text{FF}} = 21 \pm 2$ Hz.

We therefore conclude that we are observing, for the first time, ^{19}F - ^{19}F spin-spin coupling between the nuclei on different tryptophan residues in the protein.

Such coupling is termed 'through-space' coupling, since there is no inductive mechanism available. The concept of through-space coupling is well-established in small molecules²¹⁻²⁶ where the interacting nuclei are close together in space though separated by four or five σ bonds. For such through-space coupling to occur the fluorine atoms must be sufficiently close together ($<4\text{\AA}$) for there to be significant orbital overlap²²⁻²⁶. As the internuclear separation decreases to $3\text{\AA}$ coupling constants of approximately 20 Hz have been observed and further decrease in the internuclear distance leads to a sharp increase in the magnitude of the coupling constant. We conclude that two fluorotryptophan residues in *L.casei* dihydrofolate reductase must be so disposed that fluorine substituents are approximately $3\text{\AA}$ apart (approaching van der Waals contact). Since no two tryptophan residues are adjacent in the primary structure of the protein (K. Batley and H. R. Morris, personal communication), this proximity must be a consequence of the folded structure of the protein. (It is apparent in Fig. 1b that the resonance of the denatured enzyme shows no splitting.) The measurement of through-space ^{19}F - ^{19}F spin coupling constants clearly provides us with a sensitive method of detecting conformational changes in this part of the protein. In a series of complexes with substrate, coenzyme or inhibitors (alone or in combination) the magnitude of the splitting was completely unchanged, even though the chemical shifts of these two ^{19}F signals changed by up to 1 p.p.m.¹⁹. Thus, although the environment of the two tryptophan residues changes, their relative position does not.

The observation of through-space ^{19}F - ^{19}F coupling in proteins opens up an entirely new source of information on protein structure and protein-ligand interactions in solution. A systematic study of a series of selectively fluorinated derivatives of a protein could provide much valuable information on the proximity of individual residues to one another in the protein. The principle of the method could readily be extended to studies of interactions between fluorine-containing ligands and fluorine-containing proteins. The appearance of through-space coupling between a nucleus on the ligand and one on the protein is entirely feasible, and would provide unique information on the structure of the binding site in solution. Through-space ^1H - ^{19}F coupling also occurs²⁶, and would open up further possibilities for the study of protein-ligand interactions using a fluorine-containing ligand and a normal protein. It should be noted that spin-coupling constants can be detected only in the spectra of low-molecular weight proteins: for higher molecular weight proteins ($>30,000$) the short relaxation times preclude such measurements.

The information obtained from observations of through-space coupling is similar to that which can sometimes be obtained from studies of the nuclear Overhauser effect^{27,28}, and a combination of the two approaches should provide structural information on proteins in solution. We plan to explore the potential of these methods fully in our studies of ^2H - and ^{19}F -labelled dihydrofolate reductase.

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matters arising

Molecular chirality of life and intrinsic chirality of matter

BONNER *et al.*¹ stated that if racemic DL-leucine was bombarded with left-handed electrons (-1 helicity) plus their bremsstrahlung, the D enantiomer decomposed more quickly, whereas with right-handed electrons ($+1$ helicity) plus their bremsstrahlung the L enantiomer decomposed faster. In an aqueous system of D- and L-tyrosine and using only natural, left-handed β^- particles and their bremsstrahlung radiation I found⁸ similar results in 1968. Bonner *et al.*¹ referring to my experiment, wrote: "One of us has been unable to repeat Garay's original type of experiment". They used the word 'repeat', erroneously. Bonner² certainly repeated parts of my experiment for his paper but he used only bremsstrahlung radiation and left out β^- particles from the reaction mixture. This altered the situation fundamentally and therefore it cannot be considered repetition. Bonner *et al.* discussing their results, state: "We do not know whether the asymmetric degradations are caused by the longitudinally polarised electrons or by their bremsstrahlung"¹. Thus the presence of longitudinally polarised electrons are clearly important. According to our hypothesis^{8,9} and to a similar one put forward by Noyes³, longitudinally polarised electrons are responsible for stereoselective degradation. Additionally, Keszthelyi^{4,5} and Walker⁶ pointed out that selective decomposition of enantiomers cannot be caused by bremsstrahlung. Finally, in recent experiments, Darge *et al.*⁷ studied the stereoselective decompositions in a similar system. They changed the radiation source and target molecule, but did not omit β^- particles from the reaction mixture. Referring to my work they wrote that their result "is in qualitative agreement with previously reported experiments on the interaction of D- and L-tyrosine with β^- particles".

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BONNER, VAN DORT AND YEARIAN
REPLY—Garay quotes incompletely from our paper¹ on the asymmetric degradation of DL-leucine, then claims that we use the word "repeat" erroneously. We said¹, "In addition, one of us has been unable either to repeat Garay's original type of experiment with alkaline DL-tyrosine or to observe the induction of optical activity in a number of other racemic amino acid substrates as well". Neither in this quote nor in the paper to which it refers² do we claim an attempt to repeat Garay's exact experiment. In fact, we elaborate in detail on the reasons for which we modified Garay's experimental protocol, because² "we have felt some slight misgivings about Garay's experiment. In the first place the β -source was relatively weak, only about 0.36 mCi of $^{90}\text{SrCl}_2$. Second, the intended reaction was not primarily induced by the β -rays or their bremsstrahlung, but only presumed to be in some way enhanced by them. Thus the effect was noted only in alkaline, but not acidic solution. Third, the reaction was conducted in aqueous-ethanol solvent, where solvent photochemistry or radiochemistry (which would involve symmetrical radical intermediates) would be superimposed upon and might obscure any actual asymmetric β -ray or bremsstrahlung effects. Lastly, we felt that mere differences in the shapes of two ultraviolet absorption spectra were hardly the best or most convincing criteria for the generation of optical activity. For these reasons we decided to repeat Garay's type of experiments, with considerable modifications which would hopefully circumvent some of the above drawbacks." Since the Vester-Ulbricht β -decay mechanism³ which we were investigating involves β -ray bremsstrahlung, we used² the strongest bremsstrahlung source we could locate, 61.7 kCi of ^{90}Sr - ^{90}Y oxide in storage at Oak Ridge National Laboratory. To minimise other sources of ambiguity inherent in Garay's original experimental design we irradiated both racemic as well as optically-active amino acids, both crystalline and in solution, then analysed the percentage degradation and enantiomeric composition of the radiolysed samples by gas chromatography. Clearly, these experiments represented extensions of and not a repetition of Garay's original experiment. In none of these experiments was asymmetric degradation observed. Our subsequent suc-

cessful asymmetric degradations of DL-leucine with longitudinally-polarised linear accelerator electrons of both handedness¹ involved crystalline amino acid targets in a collodion matrix under high vacuum, and certainly also do not remotely approximate Garay's original experimental conditions. Nor do the recent experiments of Darge⁴ involving the radiolysis of DL-tryptophan by ^{32}P in a frozen ice matrix constitute a duplication of Garay's experiment. The simple fact is that no one, including Garay, has made an attempt to repeat his original experiment exactly, and no one has claimed to.

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Do viruses use calcium ions to shut off host cell functions?

DURHAM¹ suggests that Kamine and I² have been "misled into putting false emphasis" on the role of Mg^{2+} relative to Ca^{2+} in controlling cellular metabolism because we have been unduly influenced by the fact "that cells respond more to changes in external Mg^{2+} than Ca^{2+} ". This represents a ordinate control of metabolism and misreading of my reasons for proposing a central role for Mg^{2+} in the co-growth of animal cells. These reasons were set out at greater length previously^{3,4} and have been reiterated and expanded more recently (see ref. 5 for example). My emphasis on a regulatory role of Mg^{2+} stemmed from the observations that any of a variety of unrelated substances which stimulate the multiplication of chicken embryo fibroblasts in culture, also stimulate a singular array of reactions associated with transport⁵, energy metabolism⁷, differentiated function and macromolecular synthesis^{8,9}. This singular array was termed the coordinate

response^{3,5} and includes reactions which are independent of one another and of macromolecular synthesis. Therefore, it was necessary to invoke an intermediary agent between external signal and intracellular response with the capacity to modulate many different metabolic pathways at the same time. The only specific enzymatic steps then known to be accelerated by the stimulatory treatments were the transphosphorylation reactions of glycolysis^{7,10}, all recognised as control points in this pathway and all requiring Mg^{2+} (refs 11–13). It has also become evident that some key regulatory enzymes vary in activity with their degree of phosphorylation^{14,15} and that the activities of the protein kinases or phosphatases which determine this degree are sharply dependent on $[Mg^{2+}]$ in the physiological range. In a slightly different cellular domain, we have shown that the rates of uptake of hexoses and uridine into chicken embryo cells, which are governed coordinately by external effectors, can be controlled by Mg^{2+} , but not by Ca^{2+} , in a manner that simulates in detail the kinetics of the physiological response¹³.

It is generally agreed that over 90% of the Mg^{2+} in cells is bound, largely to membranes and macromolecules^{17–20}. Changes in configuration of the binding structures or in their microenvironment would alter the availability of Mg^{2+} for its metabolic tasks. Increasing the permeability of the cell membrane to Mg^{2+} would also tend to drive up the intracellular concentration of Mg^{2+} (ref. 21). Because the estimated concentration of free intracellular Mg^{2+} is less than that required for maximal activity of many key regulatory enzymes^{11–14} any small change would have far reaching effects²².

By contrast, the concentration of free Ca^{2+} in cells is far too low²³ to affect the regulatory enzymes of the pathways involved in the coordinate response^{3,5}. Perhaps some mechanical responses which involve proteins with a very high affinity for Ca^{2+} are under its control. But, the remarkable sequestering power of the cell for Ca^{2+} (ref. 24) would ensure that such a response would be short-lived, unlike the coordinate response which is maintained for many hours, requires continuing stimulation, and culminates in accelerated cell division.

There would seem to be some merit in Durham's suggestion that the inhibition of cell metabolism which accompanies infection by cytotoxic viruses is caused by a gross increase in intracellular Ca^{2+} following damage to the cell membrane. This follows from the observation that an internal Ca^{2+} level, $[Ca^{2+}] \geq 10^{-4} M$ interferes with key Mg^{2+} -dependent reactions in the cell^{25,26}. Beyond such pathological

effects, however, the role of Ca^{2+} seems likely to be restricted to short-term structural and mechanical responses in keeping with the pulse-like, localised nature of its fluctuations, and to the limited number of proteins which can respond to $[Ca^{2+}]$ within the physiological range of $<10^{-7}$ – $10^{-5} M$. Long-term inhibition of metabolism by Ca^{2+} , deprivation may be the indirect result of lowering free Mg^{2+} within the cell⁴.

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DURHAM REPLIES—The best current estimates of ion concentrations in resting eukaryotic cytoplasm are around $10^{-3} M$ for Mg^{2+} and $10^{-7} M$ for Ca^{2+} . This 10^4 -fold difference explains why Ca^{2+} and not Mg^{2+} is used as a short-term intracellular signal in movement, hormone action, synaptic transmission, protozoan chemotaxis, vision and bioluminescence. Powerful homeostatic mechanisms tend always to maintain very low cytoplasmic Ca^{2+} concentrations. Prolonged overwhelming of these mechanisms would produce a spectrum of changes strikingly like those produced by many lytic or transforming viruses.

Rubin and his colleagues have shown clearly that changes in extracellular Mg^{2+} concentrations can greatly affect cells. Others have shown analogous responses to extracellular K^+ or H^+ levels, and to agents that affect polyamine metabolism. Responses to extra-

cellular Ca^{2+} are notoriously variable, however, even for processes that undoubtedly involve intracellular Ca^{2+} . One reason is that eukaryotic cells conduct most Ca^{2+} fluxes across internal membranes, to and from substantial calcium reservoirs for which there are probably no magnesium equivalents. In the long term, feedback relationships between different ions tend to obscure the primary ion fluxes, so that one is probably wise not to make categorical statements about any one ion.

Rubin's statement that the cytoplasmic Ca^{2+} concentration is too low to affect the "coordinate response" is wrong. Micromolar Ca acts on adenyl and guanyl cyclases and phosphodiesterases, with consequent effects on cyclic nucleotide levels and kinase activities. It also acts on K^+ and other ion fluxes, and on DNA precursor synthesis. Rubin's implication that intracellular Ca^{2+} ions act via Mg^{2+} -dependent reactions can be only partly true.

Rubin and I agree that cell biologists frequently postulate, and then expensively seek, macromolecules to fill roles that ions can fill much more simply.

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Cholinergic link in yawning

HOLMGREN *et al.*¹ recently focused attention on the basic mechanism behind the act of yawning. They reported that physostigmine and pilocarpine induce yawning in young male rats and hypothesised that a central cholinergic link may be involved in the reflex. Our results support such a proposal.

Yawning is a characteristic sign of withdrawal from morphine in man² and monkeys³. When naloxone (0.5 mg per kg body weight), but not physiological saline, was injected subcutaneously (s.c.) into three 'ex-addict' baboons (two male and one female, 4.4–5.2 kg), 98 d after abrupt withdrawal of morphine, a low incidence of yawning (2–4 episodes) occurred within 15 min; on this occasion, other signs of long-term withdrawal were absent. Seven days later, the same baboons were again challenged with naloxone, 20 min after physostigmine (0.05 mg per kg s.c.). Although this dose of physostigmine *per se* did not elicit yawning, with each animal there was a threefold increase in the incidence of yawning.

Although a cholinergic link may indeed be involved in yawning, it should be recognised that other factors are also important. Thus, dimethyltryptamine causes

yawning after intramuscular administration to rhesus monkeys⁴ yet there is no evidence that this hallucinogen has marked effects on the cholinergic system in this species.

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URBÁ-HOLMGREN AND HOLMGREN
REPLY—In Cowan's experiments with 'ex-morphine addict' baboons, although physostigmine 0.05 mg per kg s.c. *per se* did not evoke yawning, this dose strongly potentiated naloxone-induced yawning. Perhaps a higher dose of physostigmine (0.10 mg per kg) would have elicited yawning directly even in monkeys, as it does in rats¹ and in infant guinea pigs, kittens and dog pups (unpublished observations). In infant rabbits we have had to use a still higher dose (0.15–0.20 mg per kg).

We certainly agree with Cowan that other factors are also important in yawning. The stretching and yawning syndrome induced by ACTH and MSH (quoted in ref. 1) is a well-known example. In recent experiments with (3,4 dihydroxyphenylamino)-2-imidazoline (DPI, Böhringer) we have observed that this drug, which according to Cools *et al.*² has a specific and potent agonistic activity at dopamine inhibitory receptors, in doses of 5 mg per kg intraperitoneally, elicits moderate, but statistically significant yawning in infant rats (from 9 to 15 d in age). It would be rash at this stage, to hypothesise that drugs shown to elicit the yawning act necessarily through a final common path including a cholinergic link.

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Lysogeny by f2 phage?

ZGAGA¹ claims to have produced "the first demonstration that bacteria can also contain the genetic information for the production of an RNA phage", and raises the question whether RNA phages can lysogenise their host in certain conditions. Her criteria are the "spontaneous release of

phage particles" and the "immunity" of the host to superinfection.

Davern², and Hoffmann-Berling and Mazé³ reported that *Escherichia coli* infected with RNA phage (can₁ or fr, respectively) when incubated at 32°C rather than 37°C seemed to grow normally while producing phage at a low level. The suggestion that single infected cells excreted phage without lysing³ has been challenged⁴. Davern further noted that even at 37°C phage-infected cultures showed variable degrees of lysis and the surviving cells seemed to multiply normally while producing phage indefinitely in successive cycles of subculture. Such cells, when used as host bacteria in a plating assay, did not permit formation of plaques. The latter observations have been repeated several times in our laboratory when indicator bacteria have been accidentally contaminated with phage.

The state in which bacterial cultures produce phage without lysing and are resistant to superinfection has been called 'carrier state'² or 'viral persistence'⁵ without implying any mechanism. Resistance to lysis occurs in certain physiological states of *E. coli*⁶ and resistance to superinfection (interference) has been described as a normal consequence of RNA phage infection⁷. The phenomena described by Zgaga are reminiscent of the earlier observations and raise the question whether guanidine is required to establish cultures of bacteria "containing genetic information for f2 biosynthesis".

The term 'lysogeny' implies that the viral genome is present in the host in a non-infectious form, in particular that it is integrated into the host genome⁸; until evidence for such criteria is adduced in the case of RNA phages it would be preferable to retain the original designation of 'carrier state'.

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ZGAGA REPLIES—Weissmann's objection is based on the observations of Davern¹ and of Hoffmann-Berling and Mazé², that *E. coli* infected with some RNA phages apparently grow normally at lower temperatures while producing phage at a low level. Loeb and Zinder³ showed that at 37°C f2 phage lyse the bacteria they infect. Lerner and Zinder⁴ showed, by the study of release of bacteriophage f2 from single infected cells, that at 30°C as well as at 37°C phage are released by cell lysis,

and not by excretion from intact cells. Therefore, the definition of 'carrier state' mentioned by Weissmann ("the state in which bacterial cultures produce phage without lysing and are resistant to superinfection") obviously cannot be attributed to phage f2.

Originally, 'carrier state'⁵ denotes "bacterial cultures that are persistently contaminated with phage, but from which uninfected cells could be recovered readily". Thus, the maintenance of this state depends on the presence of free phage and sensitive bacteria. To eliminate any possibility of reinfection during bacterial growth, female (*con. f2*) cells, which are genotypically resistant to f2 phage, were constructed. It turned out that F[–] (*con. f2*) bacteria produce phage spontaneously. In addition, 'carrier state' is not stable. Therefore phage-free, sensitive clones can usually be isolated by growth in the presence of phage antiserum, or by serial subcultures of single colonies⁶. On the contrary, the principal feature of 'lysogeny' is its stability. Hayes said⁶: "In practice, the existence of lysogeny should be judged by rather rigorous criteria of its stability, since interaction of some virulent phage with their hosts may superficially simulate the condition" ('carrier state'). (*Con. f2*) bacteria, once established, has been stable for 2 yr. It is stable in male as well as in female cells and quite unaffected by serial subculture of single colonies or by antiserum treatment.

The functional state and the location of genetic information for phage production in (*con. f2*) bacteria are still under investigation. But, I do not agree with Weissmann's notion that the term 'lysogeny' in particular implies, that the viral genome is integrated into the host genome. For example, in lysogens carrying phage P1, the prophage does not occupy a characteristic site, if any, in the bacterial chromosome⁷, but there is strong evidence to suggest that it is a nonchromosomal element⁸, and a part of the bacterial membrane replicatory system^{9–11}.

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reviews

Astronomy of Stonehenge

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On Stonehenge. By Fred Hoyle. Pp. v+128. (Heinemann Educational: London, 1977.) £4.50.

SOME time ago, I acted as External Examiner at another university, and wanted to interview one of the candidates. She was nowhere to be found. Further enquiries elicited that she had recently become a devotee of Stonehenge; and I had been foolish enough to select the summer solstice for my oral examinations. She had chosen to attend the more important event on Salisbury Plain. I wondered then, not for the first time, what it is about Stonehenge that sets it apart in people's minds from other megalithic monuments. This even extends to discussions of its possible astronomical significance. After all, Thom has suggested that a number of the standing stones up and down the country indicate an astronomical orientation, so why do at least half of all relevant writings concentrate on Stonehenge?

Respectable reasons can certainly be advanced—the impressive size of the monument, the constructional capabilities that must have gone into its creation, the choice of site, the span of time over which it was used and modified. I suspect, however, that part of the cause of Stonehenge's attraction, even for reputable investigators, remains its extra-scientific glamour. Typically, Sir Fred Hoyle has his own different reason for studying Stonehenge:

"Because of my interest in Stonehenge, I am often asked to comment on other ancient monuments—Avebury, the Egyptian pyramids, Mayan temples, and so forth. It usually causes surprise when I express no interest. My indifference comes from a lack of definition of the problem. Given the plan of Avebury, it would be possible to invent many hypotheses about the purpose of this monument, so many indeed that soon or later, just at random, the investigator would come on something which seemed to have a tolerable correspondence with the facts. For me, the case of Stonehenge was quite different. Thanks to the work of Mr Newham and Professor Hawkins, the problem here is defined, particularly if we go on to add, as Professor Hawkins also did, the further assertion that the purpose of Stonehenge was to predict eclipses of the Sun and Moon. The problem is then defined with severe constraints. Without modifying the fixed structure in any

way, can Stonehenge be used in a literal, practical way to predict eclipses successfully? Since the successful prediction of eclipses is a complex problem, an affirmative answer to this question would certainly not be expected unless eclipse prediction was indeed the purpose of Stonehenge. The question is so definitive, permitting no room for manoeuvre, that its investigation is quite explicit."

There speaks the Cambridge-trained theoretician: we know precisely where we are (even if it is a little harsh to imply that no other site has yet had its problems properly defined).

Hoyle's book therefore has a single, limited purpose—to examine the possibility that Stonehenge was used as an eclipse predictor—and the author deploys his considerable skill as a writer in presenting his arguments and conclusions clearly and concisely. The main body of the text, though closely argued, uses no mathematics, and should be comprehensible to a wide range of readers. (The diagrams, incidentally, are genuinely helpful.) An appendix re-defines the problem in terms of spherical trigonometry; and also brings us face to face with one of the main queries concerning astronomical orientations—namely, their statistical significance. Two types of question come under this heading. The first is this: since alignments are never perfect—due, for example, to subsequent movement of the stones and to their finite breadth—what latitude of error can be permitted when deciding whether two objects are aligned towards a particular point on the horizon? Again, if a large number of possible alignments can be found (as they can at Stonehenge), what is the likelihood that astronomically significant ones will occur by chance? Hoyle examines both these points with care. He concludes with regard to the second, that the probability of chance alignments is higher than Hawkins supposed in *Stonehenge Decoded*, but is still too small to explain all the observed alignments at Stonehenge.

The discussion of Stonehenge's use as a predictor of solar and lunar eclipses essentially follows lines that Hoyle has put forward previously, though laid out here in greater detail. In particular, he spends some time considering the vexed question of how exactly observations of objects on the

horizon might have been made. This is linked to the discussion of alignment errors; since both the Sun and the Moon show a finite disc. His argument here is ingenious. He suggests that some of the apparent spread in alignments at Stonehenge, as compared with the expected directions, is due to the method of observation that was used. As a result, evidence previously used to cast doubt on an astronomical interpretation of Stonehenge, may actually support it. It is only fair to add that some of the details of the observational procedure he outlines may be open to question. The general picture, however, hangs together very well, and leads on to an interesting speculation regarding the successive stages of construction at Stonehenge.

One of the problems in interpreting Stonehenge as an astronomical observatory is that the first structure built (Stonehenge I) seems to indicate a more sophisticated approach than the final stage (Stonehenge III). This obviously runs counter to common-sense expectations. Hoyle suggests that, in the period between the two phases of building, the method of eclipse prediction changed. Stonehenge III has an inner horseshoe of 19 bluestones, and he relates this to the Saros—the 19-yr cycle that was used in antiquity to predict eclipses. Recognition of the Saros, however, almost certainly required access to written records; whereas the analysis behind Stonehenge I could have been carried out on the basis of a simple counting system. On this interpretation, Stonehenge III does not reflect a simpler understanding of eclipses than Stonehenge I: rather, the complexity has been removed from the structure and stored in another form. A numerate culture became a literate culture.

The way we interpret Stonehenge necessarily affects what we think of the people who built it. Archaeologists currently seem somewhat less concerned about the level of abstract thought implied by a Stonehenge astronomical observatory than they were a decade ago. Nevertheless, the cultural implications continue to be controversial. In the final chapter of the book, Hoyle adds his own mite to this debate. He suggests that the (invisible) nodes of the lunar orbit, which he believes

were tracked by way of the Aubrey holes at Stonehenge, might have come to be worshipped as a powerful, though unseen, god. He therefore concludes the book by querying: "Could a distant memory of [the Sun, Moon and lunar nodes] be the origin of the doctrine of the Trinity, the 'three in one, the one in three'? I suspect so. In Stonehenge I may well be many roots of our present-day culture."

Microscopic techniques

Analytical and Quantitative Methods in Microscopy. Edited by G. A. Meek and H. Y. Elder. Pp. 276. (Cambridge University: Cambridge, London and New York, 1977.) Hardback £12; paperback £4.75.

THIS volume originates in a symposium on principles, applications and shortcomings of some techniques in modern microscopy, organised under the auspices of the Society for Experimental Biology in 1975. It consists of an introduction and twelve chapters. The topics considered are stereology, optical diffraction analysis, quantitative fluorescence microscopy, image analysis, integrating microdensitometry, scanning microinterferometry, STEM, microanalysis of various sorts, and cryoultramicrotomy.

Most of the chapters are written in a clear instructive style which is fairly easy to follow, although some of the authors have lapsed on occasion into the more easily produced review type of text. The editors have done well to cajole a panel of authors of this size into producing a reasonably uniform style and standard of presentation. The chapters seem to have very few statements to which one could take specific objection, although it is difficult for one person to critically encompass accounts of all of these topics. Each of the chapters is at such a level and of

No doubt the student who stood me up for Stonehenge will feel confirmed in her choice by these words. Those who would not wish to venture so far should, nevertheless, be grateful to Sir Fred Hoyle for setting out the case for an astronomical interpretation of Stonehenge in such a readable manner.

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a style as to make it very suitable first reading for a research student or a more senior researcher contemplating working in one of these areas of technique. Such readers will get a helpful mechanistic accounts of the methods concerned. The chapters by R. W. Horne (optical diffraction analysis), D. J. Goldstein (microinterferometry and microdensitometry) and S. Bradbury (quantitative image analysis) are particularly helpful and timely accounts.

Perhaps it should be asked how fair a picture this book gives of microscopy today. Taken together, they describe methodology that finds use at present in a small minority of research projects. The overall picture of microscopy presented in this volume is one that emphasises certain (sometimes quite isolated) specialities. It is for the most part about the iced cakes of microscopy rather than the bread and butter.

Perhaps the ultimate test of a book that is designed for the teaching of a technique is to see if it can tell the researcher whether a particular approach is appropriate for answering the questions he wishes to ask. I am not certain how many of the chapters pass this test, but there are some really excellent helpful accounts of certain microscope technologies in this book. The paperback version, in particular, is fine value.

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Insect behaviour-modifying chemicals

Chemical Control of Insect Behaviour. Edited by H. H. Shorey and J. J. McKelvey, Jr. Pp. 414. (Wiley-Interscience: New York and London, 1977.) \$24.50; £14.65.

THIS book is the eighteenth publication in the series *Environmental Science and Technology*, and deals with a subject that, because of its likely contribution to a more rational use of insecticides, is of particular

relevance to the study of the environment and the technology of its conservation. All types of behaviour-modifying chemical known to influence insect behaviour by external reception, including pheromones and plant-derived compounds, are covered and their prospects in pest management are discussed.

Publication of the book has arisen from a conference, involving many leading workers in the field, held in May 1975 at Bellagio, Italy, with participants contributing authoritative chapters on the various aspects of the subject. After a short introduction, subsequent chapters are arranged into five sections. The first deals with the nature of sensory responses to

behaviour-modifying chemicals with many examples from electrophysiological studies. The next two sections are devoted to behavioural aspects and begin with a detailed account of the various mechanisms by which insects respond to distant odour sources. The fourth section considers the role of diversity in behaviour-modifying chemicals, with the approaches to their use in insect pest management reviewed at length in the final section. Each chapter has an extensive list of references including many publications from 1975. The book has a comprehensive subject index.

The present high level of research activity in chemical control of insect behaviour may mean that this book, dealing with the subject in such depth, will soon become out of date. Already, for example, the treatment of chemoklinotaxis in chapter 5 has been revised by the author, and in chapter 9 a point is made based on a recently questioned report that the sex pheromone of *Musca domestica* consists of one compound. Nevertheless, since the authors have, with only a few notable exceptions, been cautious in drawing conclusions from the data presented and are mostly in a position to judge this data critically, the likelihood of serious reappraisal of much of the content is greatly reduced.

As the book is compiled from many individual contributions, apparently without too rigorous editing, there is inevitably some duplication of information, and in some cases the treatment of such data seems to be inconsistent. This often presents the reader, however, with the opportunity to compare the various current approaches to controversial issues. For example, the subject of kairomones is treated in several different ways, and a number of authors make different suggestions for improving definitions of the terms that have been devised to fulfil the requirements of the subject.

The prospects for insect behaviour-modifying chemicals in pest management are considered critically and without the rash claims typical of many earlier writings. In addition, a number of realistic suggestions for new approaches are made although it is asserted that more rapid progress would be made in pest management if priority could be given to fundamental research in certain listed areas.

There is no doubt that this book is to be recommended. It will be of most value to those already having some knowledge of the subject and will provide a stimulating background on which to base new research. In addition, those engaged in deciding priorities for future crop protection research and development will find this book a source of useful information and ideas.

J. A. Pickett

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Chemically transmitting synapses

Synapses. Edited by Glen A. Cottrell and Peter N. R. Usherwood. Pp. xviii+385. (Blackie: Glasgow, 1977.) £13.95.

THERE is a neatness and economy about the structure and function of chemically transmitting synapses that has given them an unfailing appeal for experimenters. The action potential, a brief all-or-nothing event, invades the presynaptic nerve terminal; the transmitter, synthesised and packaged on the spot, is released in quantal, countable packets; it combines with specific post-synaptic receptor sites which (in some situations at least) act to open or close discrete ionic channels in the membrane; the transmitter is then either metabolised on the spot by an enzyme appropriately lodged in the synaptic cleft, or it is taken back up by the presynaptic nerve for re-use. The whole thing seems to have an almost Toy-town quality.

To the extent that good science consists in reducing the confusion of a

half-understood mechanism to a series of discrete steps, this quality of our present understanding of synaptic function is a testimony to the excellence of past work in this field, and the casual observer might think that there was little left to be discovered. He would be wrong, of course, and this book, which is made up of a series of papers presented at a symposium in March 1976, highlights very usefully just those areas where mysteries still abound; in doing so, it obviously has to take for granted a great deal, and should be read as an introduction to the growing points and vague edges of the subject rather than as a coherent resumé of the present state of knowledge.

Among many interesting papers is a series of contributions about the vesicle hypothesis, written mainly by sceptics, and these constitute a very valuable source of information for students of this tangled controversy. It is a pity, perhaps, that papers by some of the ardent defenders of the vesicle hypothesis, such as Whittaker or Heuser, were not included.

Another fascinating theme that runs through several excellent papers in this volume is that of the formation and stabilisation of synapses, which is approached through studies on the synthesis and degradation of receptors (an

elegant contribution by Fambrough and his colleagues), the regeneration of synaptic connections after sectioning nerve bundles in the leech nervous system (Nicholls *et al.*), and studies on muscle denervation (Guttmann). This is surely one of the most challenging problems in neurobiology, and when we consider how badly understood is the mechanism even of the much-studied effect of denervation on the distribution of receptors in skeletal muscle fibres, it is obvious that the exploration has barely begun. The Toy-town image of the finished article seems a long way away when one is faced with the problem of how the synapse develops and regenerates.

Inevitably, in a published symposium, there are inconsistencies of style and quality. Overall, though, a very high standard is achieved, and the editors are to be commended for their choice of authors. Whether much purpose is served by including 40 pages of brief abstracts as an appendix to the main part of the book is doubtful, but this is a small criticism of an otherwise excellent book.

H. P. Rang

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Laboratory handbook on lysosomes

Lysosomes: A Laboratory Handbook. Second edition. Edited by J. T. Dingle. Pp. xiv+323 (North-Holland: Amsterdam, New York, and Oxford, 1977.) Dfl.136.00; \$55.50.

OF all the articles in the multi-volume *Lysosomes in Biology and Pathology*, edited by Dingle and his colleagues, those on techniques have been most frequently consulted. Dingle therefore decided to edit a laboratory handbook on lysosomes, which has proved sufficiently popular to justify a second edition.

The most useful chapter is a scholarly account of the properties of lysosomal enzymes by A. J. Barrett and M. F. Heath. This has been brought up to date with methods of assay and much background information. The chapter on isolation of lysosomes (R. T. Dean) follows for the most part traditional lines, using loading of rat liver with Triton, dextran, iron or gold. Methods for isolation of lysosomes from single cell types, such as leucocytes and tumour cells, are also given.

L. B. Bitensky and J. Chayen describe the histochemical identification of lysosomes at the light microscopical level, and rely on microdensitometry for quantitative results. The identification of lysosomes at the electron microscopical level is discussed by J. M. P. Schellens and his colleagues. Attractive results are obtained in various cell types by techniques for carbohydrates such as silver proteinate. Even reactions for enzymes such as acid phosphatase or β -glucuronidase can be misleading. Observations must therefore be approached with caution.

During the past five years, specific antibodies to individual lysosomal enzymes have been studied by A. R. Poole and others to monitor purity of isolated enzymes, inhibit their activity in cells and tissues and localise individual enzymes within cells.

In general, this is a fairly comprehensive account of recent techniques for lysosomes and their constituents, well illustrated and produced. It will be as welcome and widely used as the first edition.

A. C. Allison

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Applications of geomorphology

Applied Geomorphology: A Perspective of the Contribution of Geomorphology to Interdisciplinary Studies and Environmental Management. Edited by John R. Hails. Pp. xv+418. (North-Holland: New York, Amsterdam and Oxford, 1977.) \$39.95; Dfl.98.00.

THE descriptive models of landscape evolution written in the sonorous prose of the last century were debated but only a little refined over 50 years, with too little attention being paid (though there were exceptions) to the mechanics of the processes invoked in sculpting type forms of the land surface. In comparatively recent years, enormous strides forward have been made, largely by application of quantitative methods in the laboratory and in the field. The subject is becoming established as a science in its own right, and though still interdisciplinary between civil engineering, geology and geography, the applied aspects compel its inclusion in optimisation of land use, in building science, and in pedology.

The subtitle of the book truly indicates the contents, written by nine authors, each an acknowledged leader in his field and (as in the editor's introduction) able to "communicate ideas comprehensible to others who have no geomorphological knowledge".

Some authors discuss widely, as in the chapter on weathering, with applications ranging from ore deposits to geomedicine, whereas others present thorough yet succinct summaries of their discipline; a very creditable example of this is the chapter on "Periglacial Environments". The chapter on irrigation and ground-water studies demonstrates how the success of irrigation schemes would be enhanced if designed to fit the geomorphic units compiled from interpretation of the geomorphic history of the area. Processes controlled by climatic differences have less importance in Karst hydrology, as the author of that chapter makes quite clear; he also cites Sweeting's view that the study of the development of Karst landforms has probably been hindered less by the dominance of cyclic ideas than by the lack of contact of workers in the different Karst regions. From work initially conceived in a spirit of academic enquiry, there is now much of importance to the engineer involved in dam building or using limestone aquifers as reservoirs or quarries for waste disposal.

The chapter on fluvial hydrology explains the relation between some of the many variables to be measured in

comprehending the river as an integration of the catchment morphology. Man's use and modification of rivers has often proved needlessly expensive in corrective measures applied where these measures opposed the natural forces.

The same can be said of the problem of routing motorways, with examples in the chapter on soil mechanics of slopes. The degree of stability of previously stable slopes cannot yet be predicted.

It seems paradoxical in the chapter on deserts to see the stress on removal of sediments by water: though widely spaced in time, these processes are critical in terrain classification and environment.

For resource appraisal, trafficability and management problems, systems of terrain classification, with subgroups of land units, are explained using block diagrams, maps and tables to help comparison of different methods and applications.

A chapter on coastal zone planning and management includes sea defences,

off-shore banks and beach stability, and discusses how these can be affected by tourist use, by mining and by construction works.

Inevitably, the great range of style and subject matter compels dipping into the book for a synopsis of specific aspects; but the 20 pages of index are inadequate for the reader looking for specific topics across different chapters—for example, rates of rock wear or removal by different geomorphic processes. The 35 pages of references are very valuable and would have been doubly so if the page number could have been added to indicate where referred to in the text.

There are numerous maps, tables and graphs and nine half-tone plates, leavening a most valuable summary of some quite difficult branches of this global science.

H. Lister

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Differentiation in cellular slime moulds

Development and Differentiation in the Cellular Slime Moulds. Edited by P. Cappuccinelli and J. M. Ashworth. Pp. xx+317. (Elsevier/North Holland: Oxford, New York and Holland, 1977.) Dfl. 79; \$39.75.

THIS volume is a collection of papers from an EMBO workshop held in April 1977. One is grateful that it has been published so quickly, but this advantage is somewhat diluted by the way in which the book is printed. The typewritten manuscripts have been directly photocopied and greatly reduced; as a result some of the papers are either so faint or the print so small that they require hard work to read. What with the high price of the volume, the least the publishers could have done is to issue a magnifying glass with each copy, like the compact edition of the Oxford English Dictionary.

Cellular slime moulds are having a burst of popularity, and this excellent collection of papers gives one a good view of current progress. Many of the papers are mixtures of reviews and new results, some of the latter being significant advances.

To show where the main interests lie at the moment, I have made a list of the areas covered in the symposium: six of the papers are on some aspect of differentiation; five on cyclic AMP pulsations and their various effects in-

cluding their role in chemotaxis; four studies which in some way relate to problems of transcriptional and translational control of protein synthesis; three on different aspects of genetics; two on the plasma membrane; and one each on cell contact, cell division, polyamines, and modelling biochemical pathways.

One theme that runs through a number of papers is the role of cell contact on different aspects of development. J. Gross *et al.* found that cell attachment during aggregation, the rate of cyclic AMP signalling and the appearance or disappearance of certain proteins, all change at the same time. H. V. Rickenberg *et al.* show that cell contact is not necessary for the synthesis of alkaline phosphatase in cells grown in liquid culture if the suspension of separate cells is pulsed with cyclic AMP. H. F. Lodish and T. H. Alton provide evidence suggesting that at early stages when cells are separate, protein synthesis is controlled at translation, since there are numerous mRNAs present which are not translated. At aggregation, however, transcriptional control takes over and all the mRNAs are directly used to make new protein.

It is fitting that this interesting collection of papers should be dedicated to Kenneth Raper who discovered *Dictyostelium discoideum* and led the way in showing the exceptional usefulness of cellular slime moulds as experimental organisms.

J. T. Bonner

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obituary

J. E. Littlewood

PROFESSOR John Edensor Littlewood, FRS, Emeritus Rouse Ball Professor of Mathematics in the University of Cambridge, died on 6 September, 1977, at the age of 92.

He was the son of Edward Thornton Littlewood, a schoolmaster who had been 9th Wrangler in 1882. Littlewood was taught mathematics at St. Paul's School by F. S. Macaulay, later FRS, an outstandingly successful teacher. Littlewood has described his mathematical education, including his early experiences in research, in *A Mathematician's Miscellany*, and it seems to have been a far from ideal preparation for the kind of research which became his life. For Cambridge mathematicians had not yet fully appreciated the major advances on the Continent.

His director of studies, E. W. Barnes, suggested the problem of integral functions of zero order, in particular asymptotic formulae for functions with given zeros. Perhaps as a result of independent reading, Littlewood switched to 'elementary' methods for general functions and produced an important result in his first paper on the maximum and minimum moduli of any integral function of zero order, a type of result which Barnes had described in 1898 as 'a disguised truism'. However, Barnes now recognised the value of the work; one referee opposed publication violently, but G. H. Hardy reported favourably and the paper was published in 1907.

In 1911, Littlewood opened a new chapter in the theory of series with his Abel-Tauber theorem which led to his immensely important collaboration with Hardy lasting 35 years.

It covered, besides the theory of series, and in particular Fourier series, the Riemann zeta function, Diophantine approximation, the additive theory of numbers and the theory of functions.

After three years in Manchester, Littlewood returned in 1910 to a Fellowship and Lectureship at Trinity College; he became Rouse Ball Professor in 1928. During the First World War he worked as a Second Lieutenant on anti-aircraft ballistics. He retained his rooms in Trinity as Life Fellow until his death. He spent little of any vacation in Trinity, finding a different setting better for concentration on research. He believed that periods of complete relaxation were essential and often found them in music, and in rock-climbing and ski-ing.

In his younger days he served on the Councils of his College, the Royal Society and the London Mathematical Society, but from about 1935 the fits of depression which had plagued him for many years seem to have become worse. He took no part in meetings of committees or councils or in gatherings of mathematicians. However, his fits of depression were cured sometime in the 1950s, and he paid several highly successful visits to the U.S.A. after retiring.

A very high proportion of Littlewood's work after 1912 was done in collaboration with Hardy, some with Paley, Offord, myself and others. When Harald Bohr, brother of Niels Bohr, paid his first long visit to Cambridge, he and Littlewood wrote a monograph on the zeta function, but, when it was finished, they were so exhausted that they could not take it to the printers. The manuscript was used later by Titchmarsh and Ingham

in writing their Tracts on the subject. Of the papers appearing under Littlewood's name alone, the one in 1925 made a very important contribution to the problem of the coefficients of functions *schlicht* in the unit circle, a problem still not completely solved.

Although in the early days there must have been many discussions with Hardy, most of their joint work was done by correspondence. It seems that there was an unwritten agreement that Hardy could write up and publish anything based on joint work, but Littlewood only allowed me to write up parts of our joint work on condition that it appeared under my name alone as 'based on joint work.' My own collaboration with Littlewood arose out of a D.S.I.R. memorandum on the nonlinear differential equations arising in radio work, and the most important result arose out of a letter to *Nature* 120 (1927), 363, by van der Mark and van der Pol on frequency demultiplication, or rather van der Pol's interpretation of it in *Proc. Inst. Radio Engrs* 22 (1934), 1051-1086.

I used to go to Littlewood's lectures on the theory of functions; some of them are included in his book of that title, and at one time he used the galley proofs. Although he had given the same lecture two or three times before, he showed such a passionate interest, always seeing things from a slightly different angle, that his audience could not help sharing his enthusiasm.

Littlewood's display of unequalled insight, technique and power as an analyst, was recognised by honorary doctorates, medals and membership of leading foreign academies.

Mary L. Cartwright

W. H. Sheldon

DR WILLIAM H. SHELDON, Director of the Biological Humanities Center in Cambridge, Massachusetts, died there on 16 September 1977. Sheldon is the man who invented somatotyping—a classification of variations in human body structure, to which he related variations in temperament, in physical and mental illness, and in patterns of growth and aging.

Born in 1898 in Warwick, Rhode Island, Sheldon grew up amid woods and marshes. His work shows the imprint of his country background; of his mother who raised five children and

was midwife to a village; of his father, a naturalist, breeder and judge of animals; and of his godfather William James, the psychologist and philosopher. At age 10 he was working for the state ornithologist, reporting on animals of the woods and fields—observing, describing, classifying. At 15 he took pride in his ability to match judges' scorings of livestock on 100-point scales. As an adult he turned his naturalist's eye on human structure and behaviour.

After public school, Sheldon attended Brown University and the University of Colorado. From the University of

Chicago he received his Ph.D. in psychology in 1926, his M.D. in 1933. He taught at Northwestern University and the Universities of Chicago and Wisconsin. A two-year fellowship in Europe allowed him to study with Jung and to visit Freud and Kretschmer.

In 1938 he moved to Harvard, where he did much of his basic research, with such colleagues as S. S. Stevens, the experimental psychologist, and E. A. Hooton, the physical anthropologist. During World War II he served as lieutenant colonel in the Army Medical Corps. From 1947 until 1959 he was Director of the Constitution Labora-

tory at the College of Physicians and Surgeons, Columbia University, succeeding Dr G. Draper, a pioneer in constitutional medicine. He has also held research appointments at the University of California, Berkeley, and, since 1951, at the University of Oregon Medical School.

Sheldon's earliest book (*Psychology and the Promethean Will*, 1936) and his latest one (*Prometheus Revisited*, 1975) are broad, provocative and provoking schemes for merging religious humanism with biologically grounded social psychiatry. Between these publications, he worked singlemindedly to propose and refine methods for describing individual human physical structure: to develop a "biological identification tag". Best known are his primary components of endomorphy (roughly speaking, the softness and roundness of a physique), mesomorphy (heaviness of bone and muscle development), and ectomorphy (attenuation, "stretched-outness"). By measuring the strength of each component in each individual and assigning a three-part index, the somatotype, Sheldon produced a tool which comes much closer to describing and encoding the great range of varia-

tions on the basic human body plan than was possible with older, pigeon-holing typologies.

Over the years his methods moved through several revisions, toward objectivity and always seeking measures that maximise invariance, that evaluate physique not just as a current manifestation but as a lifelong trajectory. Along the way he has reported associations of somatotype with temperament, delinquency, and mental illness. His reports on physique, health, and longevity, followed in the surviving veterans of the Spanish-American war (1898), and on the later careers of 200 delinquent boys first studied 25 years ago, will be completed by his colleagues.

Sheldon's studies of some biological underpinnings of behaviour offered a much-needed counterbalance to a psychology one-sidedly emphasising learning and environment. Psychology regarded this offer doubtfully, though the term *somatotyping* found currency in labelling many sorts of physique appraisal methods, much as the term *psychoanalysis* was misapplied to all sorts of appraisal and treatment methods, and even "schools" of

somatotyping developed, like the schools of psychoanalysis (Sheldon found this comparison odious).

A keen observer, Sheldon was thoroughly convinced of the value and accuracy of his observations—an asset in a pioneer researcher, a problem to academic psychologists mistrustful of personal judgments. His irreverence for some of psychology's sacred cows, and his schoolboy's sense of mischief combined with a talent for finding just the right parallel in nature to point up a description of a human structure or action, have needed many a colleague. But Sheldon enjoyed his life. A seven-days-a-week worker, a diner at cafeterias because he could not stand the delays and pretentiousness of restaurants, an invigilator against the poisons in modern life, he was also wholehearted in his appreciation of an idea, a friend, a sparrow on his windowsill, an old coin. (Numismatists know him as the author of the standard work classifying early American cents.) His work cleared and broadened one roadway into the study of human biology and behaviour that had been an overgrown trail.

Richard N. Walker

Arthur C. Hardy

ARTHUR C. HARDY, Professor of Physics at the Massachusetts Institute of Technology and inventor of the first recording spectrophotometer, died on 31 October at the age of 81.

His specialist interests were in optics and photography, and after graduating at the University of California in 1917, he immediately became involved in photography in the first world war when he served in the American Expeditionary Force as a Commanding Officer in the Photographic Section of the U.S. Army. After the war he spent two years at the Kodak Research Laboratories before taking up an appointment in 1922 as Assistant Professor in Optics and Photography at MIT, where he spent the remainder of his professional life, becoming successively Associate Professor, full Professor and, in 1961, Emeritus Professor.

He played some part in the development of sound recording on film, while in 1930, in collaboration with Professor S. F. Brown, he developed an electric organ which was intended to reproduce the sound of any musical instrument. It is, though, with his famous recording spectrophotometer that his name will always be associated, since it is fair to say that this instrument revolutionised industrial colour measurement.

Until then, spectral transmission and reflection measurements were made with visual instruments and the observations were extremely laborious and were often impossible because of

shortage of light. The recording of a spectral reflection curve through the visible spectrum in the course of only a few minutes meant that it now became a commercial proposition for the colour industries to determine the key property of their products, namely the spectral absorption of a dye or pigment.

The instrument has really had a very remarkable history. It was developed in the 1920s at MIT and a fascinating account of how the design evolved was given by Professor Hardy in 1938. (*History of the Design of the Recording Spectrophotometer*, *J. opt. Soc. Am.* **28**, 360–364; 1938.)

A number of the MIT staff participated in the project and a major element in its success was the use of an optical attenuator, a train of polarising prisms, to balance the light reflected from the sample against the light reflected from a reference white. The photoelectric cell, which was in those days a rather uncertain device, could therefore be relegated to the role of a null detector. In more recent recording spectrophotometers, on the other hand, the measurement is usually obtained by comparing the photocurrents themselves using a ratio-recording potentiometer or similar device. Yet the Hardy instrument is still holding its own against all competitors and is still regarded by many users as the standard instrument for colour measurement.

It was produced commercially by the American General Electric Company

as the G.E. Spectrophotometer, but it might have had an early rival from Westinghouse, since in 1930 I was involved, as a research engineer with Westinghouse in Pittsburgh, in discussions about the production of a competitor. These were not, however, followed up. It is now manufactured by the Diano Corporation as the Diano-Hardy Spectrophotometer and, naturally enough, includes a number of modifications and accessories although the basic design is still the same after nearly 50 years.

A further contribution which Professor Hardy made to colour measurement was the publication in 1936 of the *Handbook of Colorimetry*, prepared under his direction by a team drawn from the staff of MIT. This was the first book on colorimetry to appear following the establishment, by the Commission Internationale de L'Eclairage, of the 1931 system of colour specification based on tables defining the colour-matching characteristics of a standard observer. The many charts and tables in the Handbook provided an excellent introduction to the CIE system and undoubtedly speeded up the use of the system for colour standardisation and as a tool in the colour industries.

Professor Hardy received a number of honours, including the Frederick Ives Medal of the Optical Society of America. He also served as President of the Society from 1935 to 1937 and as Secretary from 1940 to 1957.

W. D. Wright